

No. 5. — *The Development of the Pronephros and Segmental Duct in Amphibia.* BY HERBERT H. FIELD.¹

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I. Introduction.

THE studies upon which this paper is based were undertaken with the purpose of determining the relation which the urogenital system bears to the germinal layers in Amphibia. At the time when they were begun, especial interest in this topic had been awakened by the appearance of Flemming's paper ('86), in which the author entirely confirmed the statement previously made by Graf Spee ('84), that the system was of ectodermal origin. This view was gladly welcomed on many sides, for it was felt that an origin from this source was more in harmony with general conclusions already accepted than was the method previously advocated. Moreover, a new light seemed now to be cast on the phylogeny of Vertebrates. Under these circumstances, it appeared highly desirable that the position which Graf Spee and Flemming had taken be subjected to the test of renewed investigation on other groups of Vertebrates than

¹ Contributions from the Zoölogical Laboratory of the Museum of Comparative Zoölogy, under the direction of E. L. Mark, No. XXVII.

those employed by them. The researches of these authors had been conducted on Mammalian material only, and it was the hope of the writer to find in Amphibia a similar mode of origin for the excretory duct.

The material employed in the present investigations consisted of embryos of *Rana*, *Bufo*, and *Amblystoma*. The study of the problem was begun with *Rana pipiens* Schreb. (halecina), embryos of which had been prepared in the spring of 1884 by Prof. E. L. Mark, who kindly placed his series at my disposal. In the spring of 1889, while in Baltimore, Md., I secured an abundance of the eggs of *Rana sylvatica* Le Conte.¹ These eggs are large, measuring at the blastula stage two millimeters or more in diameter. I also found them far better for embryological study than those of other species of frogs examined. An advantage which they possess for my purpose is that the germ layers are very well separated from one another. Moreover, the body cavity appears at an early stage, making the boundary between the somatic and the splanchnic mesoderm very pronounced, both in the region of the protovertebræ and of the lateral plates.

The eggs of *Bufo* studied, *B. americanus* Le Conte, were collected during the spring of 1887, in Cambridge and in Jamaica Plain, Mass. At this time I also collected a small quantity of *Amblystoma* eggs from a pond in Jamaica Plain; but a careful search, carried on during several subsequent trips to this locality, failed to yield any more eggs.

Prof. J. S. Kingsley at this time kindly sent me from Indiana some *Amblystoma* material which he had preserved; but for the determination of many points at issue I was obliged to wait till another season offered opportunities for collection. In the spring of 1889, therefore, I made a trip to Baltimore, where I was able to collect an abundant supply of the eggs of this Amphibian, most if not all of the material collected belonging to the species *A. punctatum* Linn. In this work I was accommodated at the Biological Laboratory of the Johns Hopkins University, — a privilege for which I am under obligation to that institution. My thanks are particularly due my friend Dr. T. H. Morgan for his kind assistance during my stay in Baltimore, and for material of his collection.

I may here also express my obligations to Dr. John S. Billings, Sur-

¹ Inasmuch as the observations of European investigators have usually been made on *R. temporaria*, it is of interest to note that *R. sylvatica* Le Conte has been regarded by some systematists as a variety of *R. temporaria* (Günther, '58, p. 17). In any event, the development of the two forms may be assumed to be very similar.

geon U. S. Army, for the favor of sending me from the Surgeon General's library in Washington a number of papers to which I should otherwise have been unable to gain access. I am further indebted to Mr. Samuel Garman and to Mr. G. H. Parker for the revision of my proof-sheets, and for suggestions during the progress of my work. Mr. Parker also read the earlier portions of my manuscript.

The material was prepared by ordinary histological methods; but inasmuch as many of the hardening reagents and stains which I tried gave thoroughly unsatisfactory results, I may state in brief the treatment which proved most successful. The embryos of both *Rana* and *Bufo* can be satisfactorily killed in Kleinenberg's picrosulphuric mixture; they can then be successfully stained in Orth's lithium-picrocarmin. The object should be exposed to the action of the stain as long as possible, care being taken to guard against maceration. In order to accomplish this purpose, it has frequently proved advantageous to stain the object twice, removing it after the first staining to strong alcohol. In passing the stained object through grades of alcohol, it is important to keep a little picric acid dissolved in the several fluids in order to prevent the alcohol from extracting the yellow stain from the specimen. Embryos treated in this way show a very effective double stain. The nuclei are bright carmine, contrasting with the yellow color imparted by the picric acid to the yolk spherules among which they are found. As a killing reagent, Merkel's fluid also gives good results. It should be followed by Kleinenberg's hæmatoxylin, and the decolorizing should be watched with care.

With *Amblystoma* the best method of treatment is that with Fol's chromic-osmic-acetic mixture, followed by Czokor's cochineal. The picrosulphuric mixture followed by picrocarmin, as recommended for *Rana* and *Bufo*, is also of service.

It is usually best to stain on the slide; and, in my experience, satisfactory results with hæmatoxylin can very rarely be reached by staining *in toto*.

II. Descriptive Part.

In the following account of the development of the pronephros and segmental duct, I shall first treat these organs descriptively. For this purpose, I shall take up in succession *Rana*, *Bufo*, and *Amblystoma*, and shall describe selected stages in the development of each. This account will be followed by a general discussion of nephridial organs, in which the results of other investigators will be reviewed.

A. *Rana*.

STAGE I.

Plate I. Figs. 1-3.

At the first stage which I shall describe the embryo has departed only a little from the spheroidal form presented by the egg during segmentation. The medullary plate is widely open, its lateral margins being only slightly elevated above the general surface. At the hinder end of the medullary plate the blastopore is plainly visible. An idea of the external form of the embryo can be gained by reference to Goette's figure of Bombinator ('75, Taf. III. Fig. 41), or to van Bambeke's of the Axolotyl ('80, Pl. XII. Fig. 9). In water of 15 to 18° C. eggs of *R. sylvatica* reached this stage in about sixty hours after fertilization; the eggs of *R. halcina* develop somewhat more slowly.

The general relations of the germinal layers at this stage are shown in Figure 2. The ectoderm consists of two distinct layers (Figs. 3 and 7, *ec'drm.'* and *ec'drm."*). Except in the region of the medullary thickening (*la. med.*), which is produced by a proliferation of the deeper of these two layers, the ectoderm is nearly uniform in thickness. The two layers present slightly different histological characters. In the outer layer (Figs. 3 and 7, *ec'drm.'*) the cells are large and columnar, and their external surfaces project as rounded eminences, giving a roughly granular appearance to the surface of the embryo. Each cell contains scattered pigment granules, which are especially massed along its external face. Small yolk spherules (*sph. vt.*) are present in considerable numbers. The cells of the deep layer (*ec'drm."*) are smaller than those of the outer, and are somewhat flattened. The pigment granules are scattered throughout the cells of this layer, without showing special accumulations. The yolk spherules present the same appearance as those of the superficial layer.

The entoderm and yolk cells (Fig. 2, *en'drm.* and *cl. vt.*) form the great mass of the interior of the embryo. The wide lumen of the gut traverses the dorsal portion of this mass. The chorda (*n'cl.*) has the form of a longitudinal ridge, imperfectly cut off from the entoderm below, and in contact with the medullary plate above. A single cell layer (*en'drm.*) on each side of the chorda forms the dorsal roof of the intestine. As this layer passes out laterally, it increases in thickness, becomes several cells deep, and finally merges in the mass of large yolk cells (*cl. vt.*) lying ventral to the intestinal cavity. All the cells of the entoderm contain large yolk spherules. Pigment is present in considerable quantity in the

cells bordering the cavity of the intestine; elsewhere it occurs only as scattered granules.

At this stage two plates of mesoderm (Fig. 2, *la. ms'drm.*) extend out laterally, one on each side of the chorda, and pass ventrally around the mass of yolk cells to be united in the median line below. Each plate is thickest (Figs. 1, 3, at *la. pr'vr.*) next the notochord; as it passes outward, it becomes thinner. Before the ventral surface of the embryo is reached, it is reduced to a layer two cells thick, representing the somatopleure and splanchnopleure (*so'plu.* and *spl'plu.*) of this region. The cells of the mesoderm are in general smaller than those of the yolk-entoderm. The yolk spherules which they contain are also somewhat smaller than those in the entoderm. Pigment is rarely present except in the form of scattered granules.

In the foregoing account of the relations of the germ layers the description refers in the main to the typical condition, realized in the middle trunk region; in this and in subsequent stages modifications occur in the head and tail regions. These special conditions are of no consequence for the present purpose.

There are certain histological characters, to which allusion has already been made, which may serve as criteria for distinguishing the germ layers. The most satisfactory of these is the size of the yolk spherules. As I have indicated, the spherules are largest in the entoderm and smallest in the ectoderm; in the mesoderm they are of an intermediate size. Measurements of spherules from the three layers in the region of the future pronephros gave the following results: entoderm, mean diameter of spherules, 8μ ; mesoderm, mean diameter, 5μ ; ectoderm, diameter rarely exceeds 3μ . Excluding the head and tail regions, these dimensions represent, I believe, fair averages for the whole body. The distribution of pigment affords another criterion for distinguishing the layers. In the superficial ectoderm, the pigment (Figs. 3, 7) is massed along the external surface of each cell. In the deep ectoderm, it is present in considerable quantity, but is scattered throughout the cell. Except in certain specialized regions, there is little pigment in either mesoderm or entoderm. I have also noted the differences in the mean sizes of the cells: the yolk cells are in general the largest, and those of the ectoderm the smallest, the mesodermal cells being of intermediate size. The great variability of this character prevents its having much weight, however, in determining to which of the three layers a given group of cells belongs.

I shall now consider in greater detail some of the modifications which

the mesoderm exhibits, particularly such as occur in the region where the pronephros is subsequently developed. For this purpose I have selected two embryos of Stage I. which show slightly different conditions. The account will first relate to the specimen which is shown, by the less differentiation of the medullary plate as well as by other features, to be the younger. This embryo measures 2.31 mm. in length. In following a series of cross sections forwards, the three germ layers become apparent at about 0.35 mm. from the posterior end, or a short distance in front of the blastopore. Here the structure of the mesoderm is rather obscure, since in a transverse section of the animal this layer is cut obliquely. The condition, however, is here nearly the same as that which I am about to describe for a more anterior section.

Figure 3 represents a section of this embryo 0.91 mm. from the posterior end. On the ventral side of the embryo the mesoderm consists of two layers, each of which is only a single cell in thickness. These two layers, which represent somatopleure and splanchnopleure, are separated by a narrow space, the *cœlom* (*cœl.*). In the lower left-hand corner of the figure, the beginning of this two-layered condition of the mesoderm can be seen. On following the mesoderm towards the dorsum, it becomes gradually thicker. In the mesoderm of this region there is found an extensive cavity (*cœl.*), which is usually irregular in outline, and might be mistaken for a *wholly* artificial condition. That the two layers were once in contact is shown by the correspondence of outline on the two sides of the space. The separation along this line is so regular, however, in successive sections, and recurs so frequently in other embryos, that the cavity must be regarded as an artificial expansion of an already existing split, rather than as an indifferent rupture of a solid mass of cells. In many sections of this embryo it is easy to trace a line of division reaching from the ventral cavity (*cœlom*) to the large lateral cavity just described. This, then, represents a portion of the *cœlom* (normally, I believe, closed), and the layers of mesoderm on the two sides of it are consequently somatopleure and splanchnopleure. The mesoderm in this region, as I have stated, is several cells deep. Along the inner and outer edges of the wedge-shaped plate of tissue constituting the mesoderm of either side, the cells, except where artificial ruptures occur, are in close contact, and form an epithelial lamella. The central portion of the plate, where this is more than two cells in thickness, contains cells of a more rounded shape, which do not form definite rows, but which are closely applied to the outer layer, — a condition which becomes quite evident when the *cœlom* is artificially enlarged.

The somatopleure of this region, then, is a layer at least two cells in thickness. The splanchnopleure, on the other hand, in this as in later stages, consists of a layer one cell in depth, extending from the ventral surface of the animal to the protovertebral plate.¹ Naturally no sharp line of division can be drawn at this stage between the protovertebral plates and the adjacent portions of the lateral plates. In the section under consideration, the protovertebral plate is rather compact, and it is difficult to indicate with certainty the boundary between the somatic and splanchnic layers. A study of this portion of the mesoderm, however, has convinced me that the cœlom (*cœl.*¹) is already outlined, and lies in such a position as to leave only a single layer of cells dorsal to it, — a condition which is perfectly evident in later stages. It is indicated by such a distribution of pigment as is seen to the right in Figure 3.

On following the series of sections farther towards the head, a constriction of the mesoderm appears beneath the lateral margin of the medullary plate, and the open cœlom is continued into the protovertebral plate. In a section 1.2 mm. from the posterior end the somatic and splanchnic layers are each but one cell thick in the region of the protovertebral plate. The cells of the somatic layer, which in the protovertebral portion are of a high columnar form, become tile-like beneath the pronounced lateral thickening (compare Fig. 1, *cras. gn.*) of the medullary plate. The somatopleure immediately lateral to the medullary plate is rather thick, and becomes thinner both towards the median dorsal and median ventral lines. The regularity of the bounding walls of the body cavity in this region, and the occurrence of a space where no other signs of distortion are apparent, lead me to believe that the separation of the two layers of mesoderm is here perfectly normal, and not, as in more posterior regions, an artificial separation of two closely applied lamellæ.

It is, in general, very difficult to observe karyokinetic conditions in mesodermal or yolk cells, owing to the presence of the large and numerous yolk spherules; but I am reasonably certain that I have observed cells in the somatopleural thickening, dividing in a plane parallel to the surface of the layer; i. e. the cells were dividing in such wise as to increase the thickness of the layer.

In a section 1.32 mm. in front of the posterior end, the lateral portion

¹ The differentiation of the protovertebræ has not yet begun in this region, and I shall designate the thick masses of mesoderm on each side of the chorda as protovertebral plates.

of the medullary plate is greatly thickened, and the lateral plates are thereby wholly cut off from the protovertebral plate. The thickening of the medullary plate is the hinder portion of a considerable ganglionic mass, which is the basis for the subsequently differentiated ganglia Gasseri, acusticum, and nodosum.¹ The somatopleural thickening may be traced to a point about 80μ farther forward, where the body cavity is no longer expanded. The relations of this thickening to the nephridial organs will be discussed in connection with Stage II. (page 211).

In a slightly older embryo, measuring 2.34 mm. in length, the condition of the mesoderm is nearly the same as in the one last described. The somatic layer shows a marked thickening (Plate I. Fig. 1, *cras. so'plu.*), which is greatest immediately lateral to the protovertebral plate. An anterior coelomic chamber is also present. The anterior limit of the thickening is situated, as before, about 0.1 mm. in front of the hinder end of the enlargement which is destined to give rise to the cranial ganglia. The thickening (Fig. 1, *cras. so'plu.*) of the somatopleure is slightly more pronounced than in the younger embryo.

The results of this study may be summarized as follows. There exists already at this stage a slight somatopleural thickening, which is maximum along a line immediately lateral to the protovertebral plate. This thickening is associated with a local expansion of the coelom. It is most pronounced in the region directly posterior to the cranial ganglionic mass. Posteriorly it is lost in a general lateral thickening of the somatic layer. The location of the thickening corresponds closely with the region in which the pronephros and segmental duct later arise. Whether we have in this thickening the first rudiment of the excretory system will be discussed in connection with Stage II.

¹ I may here note that I have been able to make out for the series of spinal and cranial ganglia in *Rana*, *Bufo*, and *Amblystoma* an origin not unlike that described by Beard ('88, pp. 166, 183) in *Selachii* and *Aves*, and by Schultze ('88, p. 349) in *Rana*. The ganglia are developed from the ectoderm at the lateral margins of the medullary plate (Fig. 3, *fud. gn. spi.*). The differentiation of the ganglia is already apparent before the neural tube is infolded. A spinal ganglion does not arise as an outgrowth from the neural tube, nor as a separate thickening of indifferent ectoderm, but is differentiated from a first rudiment (*Anlage*) common to it and to the neural tube.

STAGE II.

Plate I. Figs. 4, 5. Plate II. Figs. 13, 14.

This stage includes embryos with a distinct medullary groove, the edges of which, however, have not yet fused to form a complete neural tube. Several protovertebræ can be distinguished.

In treating of the structure of the pronephros in this stage I shall first consider two embryos, which, judging from external appearances, seem to have reached the same stage of development. These embryos are about as far advanced as the one figured by Hertwig ('83, Taf. V. Fig. 6). In both the medullary groove is widely open. They are about 2.5 mm. long, and have been sectioned, one transversely, the other frontally.

Following the series of cross sections forward from the tail end, and comparing them with those of the preceding stage, the changes which have occurred will be apparent. In the posterior region, the mesoderm, as it passes outward and downward from the chorda, tapers much more rapidly than in the earlier stage. Even as far posteriorly as a few sections in front of the blastopore, this condition can be observed; and, in a section 0.72 mm. from the posterior end, the thick central mass of mesoderm, the protovertebral region (Fig. 4, *la. pr'vr.*), has a triangular outline in cross section, and is readily distinguishable from the lateral plate (*la. l.*), with which it is continuous at its outer angle. The protovertebral plate consists of an outer epithelial layer and a central mass of cells. It is the former which is prolonged into the lateral plates. Each of these is here in general only one cell deep. Between somatopleure and splanchnopleure a few scattered cells occur, which can be assigned only with difficulty to either layer.

At 0.96 mm. from the posterior end the hindermost protovertebra visible in cross section can be distinguished. Between this point and the ganglion nodosum four protovertebræ are to be observed. Passing farther forward, it is difficult to assign boundaries to the protovertebræ. There is certainly one which is partially broken up into mesenchymatic tissue.¹ Still farther forward the series of the protovertebræ is continued by mesenchyme of a yet looser structure. Inasmuch as I have

¹ I use this expression merely as descriptive of tissue of a certain histological character, quite independently of its origin. Indeed I am convinced, from observations which appear in the sequel, that not merely the head mesenchyme, but also much of that in the trunk, arises in relatively late stages from mesodermal tissue, substantially in accordance with the account of Balfour ('78, pp. 107 *et seq.*), which has recently found champions in Ziegler ('88) and others.

reached no conclusions respecting the number and position of the head somites, and since great diversity of opinion exists in the accounts to be found in the literature, I shall make no attempt to number the protovertebræ with which I shall have to do in any other way than by beginning with the most anterior that is readily distinguishable. Disregarding, then, the one which is wholly broken up into mesenchymatic tissue, somite I. lies in the same transverse plane as the fundament¹ of the ganglion nodosum, and extends backward to the hinder end of that structure. This protovertebra also shows signs of extensive conversion into mesenchyme, although part of it at a later stage undergoes muscular differentiation. Somite II. is the first of the series of well developed trunk protovertebræ. In the specimen under consideration somites I. to VI. are already differentiated.

As I have stated, the somatopleure in the middle of the trunk consists of a layer one cell deep, to which a few loose cells lying between it and the splanchnopleure may possibly also be assigned. In the region of somite IV. the somatopleure becomes thickened. The thickening is greatest at the level of the lower margins of the protovertebræ (compare Plate II. Figs. 15, 16), and tapers both dorsally and ventrally. It is to be remarked in this connection that the protovertebræ are not yet fully separated from the lateral plates; but that in cross sections through the middle of a somite, — i. e. midway between the anterior and posterior faces of a protovertebra, — the coelom can be traced to the dorsal margin of the protovertebra, and furthermore that the somatopleure and splanchnopleure are seen to be continuous with the somatic and splanchnic layers of the protovertebræ. The somatopleural proliferation extends forward as far as the anterior face of somite II. The cells in the thickening have a columnar shape, and are at least two deep. In some sections I have observed, in addition, a third row of thin cells next the body cavity. Near the ventral limit of the thickening a nearly horizontal line of division in the substance of the thickening can be observed. When seen in cross section, this line is slightly concave above. It is here that ruptures produced by artificial causes are likely to occur, and the line thus indicated marks, I believe, the lower limit of the pronephros. The somatopleural thickening is the fundament of the pronephros, and I shall call it in the following pages the

¹ In the following pages I shall use the word *fundament* as an equivalent of the German expression *Anlage*, the term *fundamentum* having been adopted as the basis for the lettering of the figures of such structures in the "Contributions" from this Laboratory.

pronephric thickening. The dorsal portion of the expanded body cavity is the pronephric chamber.

The question whether the somatopleural thickening described in Stage I. be an early condition of the pronephric thickening is only to be answered by considering the fate of the former. Behind somite IV. this early thickening wholly disappears, and the one which is seen at a later stage is an independent formation. This conclusion is justified by a comparison of Figure 4 (Plate I.), showing the somatic layer to be only one cell thick in the posterior region of an embryo of the present stage, with Figure 3, which shows a two to three layered somatopleure (*so'plu.*) in a somewhat more anterior region of an embryo of the next younger stage. In the region of somites II., III., and IV., however, the somatopleure never wholly thins out; but the thickening is here moulded into a more definite form, and becomes the fundament of the pronephros. To my mind, it is as if the mesoderm, in the process of becoming thinner, was overtaken by the necessity of affording material for the formation of the pronephros and duct, and, as a matter of physiological economy, used for that purpose an accumulation of cells already present. Indeed, from the form of the thickening in anterior portions of the embryo, I am disposed to regard the differentiation of the pronephric thickening in this sense as having begun already in Stage I.

The corresponding series of frontal sections shows five well developed protovertebræ, representing somites I.-V. (Plate II. Figs. 13, 14). A mass of mesenchymatic tissue in front of somite I. is doubtless the remnant of the rudimentary anterior protovertebra observed in the series of cross sections, and behind somite V. the differentiation of a sixth is faintly indicated. Above the level of the lower border of the chorda the protovertebræ are sharply marked off from one another, and the somatic layer is relatively thin. Near their ventral margins, however, the successive protovertebræ are in close contact, and the somatic layer shows a pronounced lateral thickening (Fig. 13, *cras. pr'nph.*).

On passing ventrally to the region of the lateral plates, the interprotovertebral constrictions vanish. Since frontal sections, however, do not here cut the layer of mesoderm perpendicularly, certain sections in the series show a distinctly segmented splanchnic layer, while the somatic thickening in the same frontal plane is unsegmented. Farther ventral there are no traces of segmentation in either layer. Here the splanchnopleure (*spl'plu.*) uniformly consists of a single layer throughout its entire extent. The somatopleure facing the ganglion nodosum, and also that in the posterior region, is thin; but in the anterior portion of

the trunk, immediately behind the ganglion nodosum, there is a marked thickening (*cras. pr'nph.*), which ends abruptly in front, but gradually thins out into indifferent somatopleure behind. This thickening is distinctly present through a length of 0.5 mm., which is slightly greater than the extent of protovertebrae II., III., and IV. Still farther ventrally, the antero-posterior extent of the thickening is much diminished, the reduction taking place from both ends, so that in passing ventrally the region in which the structure is last visible is situated approximately beneath protovertebra III.

Another pair of embryos, one of which was 2.5, the other 2.6 mm. in length, presented a condition of the pronephros somewhat more advanced than that just described (Plate I. Fig. 5). In these embryos the lips of the medullary fold in the most advanced region were in contact, but had not yet fused. The anterior limit of the pronephric thickening was the same in position as in the younger pair of embryos, lying near the middle of somite II. A study of the arrangement of the nuclei in this region made it evident that there were at this stage in general three layers in the thickening. The innermost of these is the thinnest, and is destined to become the peritoneal covering of the pronephros; the other two represent the two walls of the pronephric pouch, soon to be described. The pronephric thickening in the region of the anterior face of somite IV. is shown in Figure 5. The section gives a somewhat false impression as to the somatic layer of the protovertebra, unless the relation of the section to the successive somites be borne in mind. The considerable thickening which this layer apparently undergoes on passing into the protovertebra is due to the circumstance that the section here passes obliquely through a portion of the anterior wall of somite IV. Sections through the middle of a protovertebra show a gradual thinning of the somatic layer as far as the dorsal angle of the mesoderm (compare Plate II. Fig. 15, which is a cross section of the following stage), where this layer is almost pavement-like. The pronephric thickening extends rather farther posteriorly than in the former pair of embryos, and while it is manifestly difficult to set a limit to the structure, I am confident that the thickening extends into somite V. This posterior extension of the thickening is to be regarded as the fundament of the *pronephric*, or, according to the later nomenclature of Balfour, the *segmental duct*.

The corresponding series of frontal sections shows six well differentiated protovertebrae, representing somites I.-VI. The same group of cells which I interpreted before as the last remnant of a rudimentary

anterior somite is still present, and a few more posterior protovertebræ are in process of formation. Frontal sections just ventral to the chorda are very instructive. By following through a series of these, an idea can be had of the successive changes which take place in passing from the protovertebræ to the lateral plates, — a region of prime importance for problems respecting the development of the urogenital organs. In sections approximately tangent to the chorda at its ventral border (compare Fig. 5), the plane of the section passes through the ventral floor of the protovertebra, and cuts the somatic mesoderm near the place where the protovertebra passes into the lateral plate. The body cavity is expanded in the anterior part of the trunk. The mass of tissue on the median side of the body cavity appears very broad, owing to the circumstance that the plane of the section, as before noted, lies in the floor of the protovertebra. The somatic layer is several cells thick, and very compact in structure, owing to the fact that the section passes through the dorsal margin of the pronephric thickening. In following the series of sections farther ventrally, the boundaries between the segmental constituents of the pronephric thickening become indistinct; and in a section 90μ farther ventral they have wholly vanished. This section, however, still shows traces of segmentation in the splanchnic layer, which is here reduced in thickness, the plane of this section having passed ventral to the floor of the protovertebra. Still farther ventrally the segmentation of the splanchnopleure likewise vanishes, and finally the pronephric thickening gives place to undifferentiated somatopleure. I have looked in vain for prolongations of the body cavity into the pronephric mass at this stage. I believe that the pronephric thickening is to be regarded as a solid proliferation of the somatopleure, in which, however, the somatic layer of the protovertebræ takes some part.

STAGE III.

Plate I. Fig. 6. Plate II. Figs. 11, 12, 15-17.

In embryos of this stage the medullary canal is wholly closed, the fundaments of two pairs of gills are present, and the auditory vesicle consists of a shallow depression of the deep ectoderm.

The pronephric thickening has now begun to assume a more definite form, and during this stage becomes converted into a tubular organ. I shall first consider the structure as seen in a series of cross sections from an embryo measuring about 2.7 mm. in length. Figures 15 to 17 are from this series. The anterior end of the pronephric thickening is

located in somite II. The plane of the section from which Figure 15 was drawn passes somewhat behind the middle of this somite, so as to show the location of the constriction between the protovertebrae and the lateral plates. In the middle of the somite, the arrangement of the cells composing the pronephric thickening appears to be that of a fold in which the layers are in close contact. The thickening is composed of three layers of cells, and it is possible to trace the somatic layer of the protovertebra into the outer layer of the thickening. The lateral indifferent somatopleure is continuous at the ventral border of the thickening with the inner or thin layer which lies next to the body cavity. Near the upper border of the thickening this inner layer appears to be folded abruptly on itself to form the middle layer of the thickening. The middle and outer layers are continuous with each other distally, i. e. ventrally.¹ This anterior knob of the pronephric thickening (Fig. 15, *fund. neph'st.*¹) is the fundament of the first nephrostome, a later stage in the development of which is shown in Plate III, Fig. 18 (*neph'stm.*¹). In Figure 15 the three layers are indicated by the arrangement of the nuclei. Of these the two outer form the fundament of the first nephrostomal tubule. The innermost layer represents the underlying peritoneum. In the region between somites II. and III., it is impossible to distinguish definite layers in the thickening.

On entering somite III., the pronephric thickening has a far greater breadth, and it consists of three layers, the meaning of which is to be understood by a comparison with the condition in the region of the first nephrostome, just described.

In somite IV. (Fig. 16) a division of the thickening into a dorsal and ventral part is indicated, near the termination of the dotted line (*cras. pr'neph.*). The dorsal part is the fundament of the third nephrostome, and the ventral part represents the anterior portion of the segmental duct (more properly, common trunk, see page 228). The ventral portion of the thickening can be traced backwards from this point through a distance of about 0.37 mm. Figure 17 is drawn from a section through a region near the anterior boundary of somite VII., and shows

¹ The correlative terms *distal* and *proximal* are so frequently employed by German writers as synonymous respectively with *posterior* and *anterior* that it seems advisable to allude to the fact that they are not used in the present paper in that sense, but invariably with their primitive signification: thus, the distal portion of a process is that part which is most remote from the point of attachment, whether the structure project anteriorly or posteriorly, medially or laterally, dorsally or ventrally.

the thickening (*cras. pr'nph.*) near its posterior termination. The mass is evidently a thickening *in situ* of the somatopleure. On either side of the fundament of the segmental duct the somatopleure is one cell thick, whereas in the fundament itself it is two or three cells in thickness. If the additional cells arose by a free backward growth from the anterior pronephric mass, we should expect to find them lying on the external face of a continuous somatopleural layer. But, as a matter of fact, no such continuous inner layer exists; on reaching the thickened region, the somatopleure merely becomes several cells in thickness, the outer cells presenting really a somewhat more compact condition and a more linear arrangement than the inner ones.

The constrictions between the protovertebræ and the lateral mesoderm can be distinctly made out only in intersegmental regions. As is shown in Figure 15, between somites II. and III. the level of the constriction is immediately dorsal to the nephrostomal portion of the pronephric mass. In the region between somites III. and IV. the division occurs at a corresponding position. This series of sections shows no sharp separation between protovertebral and lateral mesoderm posterior to somite IV., the protovertebral plate being here only partly broken up into successive metameric blocks, which do not as yet possess sharp ventral boundaries.

In frontal sections, the pronephric thickening shows a similar condition (compare Figs. 11-14) to that which obtains in the case of the embryo described under Stage II. (page 213), the most noticeable difference being an increase in the thickness of the pronephric mass. The longitudinal extent of the thickening corresponds approximately to that of five somites, though the posterior limit is of necessity somewhat uncertain. The posterior portion has every appearance of having arisen in the same way as the part lying beneath somites II., III., and IV. The latter, however, represents, as we have seen, the future pronephros; the former is the fundament of the segmental duct.

In an embryo slightly older than those last described, the evidences of an incipient canalization of the pronephric system are more pronounced. In the region of somites II.-IV., the two outer layers of the pronephric thickening are separated from the peritoneal layer by a distinct line of division. In the intersegmental regions, the outline of these two layers is that of an elongated ellipse, the nuclei being disposed, for the most part alternately, on either side of its major axis. The significance of this distribution becomes apparent on studying later stages, in which a lumen

has appeared in the organ. It is then found that the lumen occupies the position of the major axis of the ellipse, and that the nuclei of the bounding cells lie close to the interior surface of the wall. If a tube so constituted be compressed laterally, so that the lumen wholly disappears, it is evident that the cells of the opposed walls would be likely to accommodate themselves to one another so as to present an alternate arrangement of their nuclei.

Opposite the middle of a somite, the relations are somewhat different. Here the two layers of what I shall hereafter call the pronephric pouch do not remain confluent at its dorsal extremity, but separate, the outer becoming continuous with the somatic layer of the protovertebra, the inner with the deepest layer of the thickening, and thus finally with the lateral somatopleure. In this region the body cavity can be seen to project for a short distance between the two layers of the pronephric pouch, as shown in Plate I. Fig. 6, *cæl.* This figure demonstrates very clearly the relations of the pouch to the lateral mesoderm and the overlying somites.

In the case of the younger set of embryos which have been considered in this stage, it will be remembered that the boundary between the lateral mesoderm and the protovertebrae was evident only in inter-segmental regions. In the somewhat older individual now under consideration, the constrictions between these two portions of mesoderm have advanced into segmental regions as well; so that now, for the first time, the precise relations between the fundaments of the nephrostomes and the protovertebrae lying above them can be accurately determined. The last remnant of the communication between the protovertebral cavity and the body cavity I shall call the communicating canal, following in this the nomenclature of Renson ('83). The section shown in Figure 6 passes through this canal (*can. comm.*), and it is to be especially noted that the constriction between the somites and the lateral plates takes place dorsal to the region of communication between the pronephric system and the body cavity. Immediately dorsal to the pronephros, the somite sends out a lateral fold of the somatic layer, which is destined to form the capsule of the pronephros, to which I shall have occasion to refer in later stages.

In somite IV., the division of the pronephric mass into a dorsal and ventral part is faintly indicated, but the dorsal part shows no trace of the lumen which is destined to become the third nephrostome. In this embryo, the constrictions between the protovertebrae and lateral plate have advanced into more posterior regions. In somite V. the constrict-

tion occurs immediately dorsal to the fundament of the segmental duct, which, as I have shown, is continuous anteriorly with the ventral half of the thickening appearing in somite IV. A series of measurements from the dorsal median line shows that the ventral portion of the pronephric thickening remains at a nearly constant level, so that the protovertebrae must reach a somewhat more ventral position in the posterior region than in somites II.-IV.

Figure 11 (Plate II.) represents a frontal section through the dorsal part of the pronephric pouch in one of the oldest embryos of this stage. It shows the course of the earliest fundaments of the three tubules which emerge from the somatopleure beneath protovertebrae II., III., and IV. The most anterior outgrowth, arising in somite II., inclines outward and backward into the region of somite III.; the second outgrowth proceeds from its origin beneath protovertebra III. directly outward; and the third outgrowth inclines forward, so that its distal extremity also lies in the region of somite III. As the review of the previous stages has shown, these fundaments of the tubules have *not arisen as separate outgrowths* from the somatopleure, but have been differentiated from the originally continuous pronephric thickening, the three fundaments being confluent distally.

In this section the nuclei are abundant along a central band, but scarce or wholly absent in peripheral parts. This peculiar arrangement becomes intelligible when we consider that the plane of the section passes almost tangentially through the curved dorsal wall of the pouch. As we have seen in transverse sections, the nuclei lie close to the inner lumen of the pouch; it is therefore only in the deeper central parts of the section that they are encountered. In a section 0.03 mm. farther ventral (Fig. 12), the lumen of the pouch can be made out, though it is not conspicuous. It is difficult to say whether at this stage the lumen is continuous throughout the whole structure. In many embryos the evidence of such continuity seems indubitable; whereas in others, apparently quite as far advanced in other respects, the lumen seems to consist of unconnected portions. In some instances where no trace of a separation of the walls could be seen, a line of pigment indicated the position of the lumen. Occasionally I have met with a distinct prolongation of the body cavity into the pronephric mass. This condition has been most frequently encountered in the case of the fundament of the first tubule. I am not, however, inclined to place much weight on such observations as proving the claim that the lumen of the pronephros forms as an ingrowth of the coelom proceeding from the nephrostomes and advancing into the duct.

On the contrary, the lumen is already potentially present, as shown by the arrangement of the nuclei before any actual separation of the walls occurs. I am of opinion that, in the cases referred to, the separation is largely artificial, and that the ruptures take place most frequently at the nephrostomes for the reason that the walls, which elsewhere form a closed ring, here have in cross section the form of a sharp re-entrant angle bordering on a large open space. It is evident that in the former region the walls would be less liable to be torn apart in the preparation of the material than in the latter. In general, however, it must be admitted that the development of the lumen, like that of the system as a whole, actually advances from anterior to posterior regions.

The fundamentals of the three pronephric tubules shown in Figure 11 are not to be regarded as outgrowths from the somites. They are, it is true, very closely related to the segments in their arrangement, but, as transverse sections prove (Plate I. Fig. 6, and Plate II. Fig. 15), they lie wholly ventral to the lower boundaries of the protovertebrae. The frontal section figured (Fig. 12) was chosen for the reason that it was the one which indicated most precisely the course of the fundamentals of the three tubules. The plane of the section is parallel to a tangent to the dorsal margin of the structure, and passes only a little below that margin, not through the nephrostomes. These begin in a more ventral unsegmented region.

In the oldest embryos of this stage, the fundamen of the duct has developed very rapidly. Anteriorly, it has in cross section a distinctly elliptical outline, and its cells have, with reference to the major axis of the ellipse, the same arrangement that I have described for the inter-segmental regions of the pronephric pouch. On following the structure backwards, this distribution becomes less and less obvious, until the cells seem to have no definite arrangement. In this region the fundamen of the duct is in far more intimate union with the somatopleure than was the case in anterior somites. In the region of somite IX. the last trace of the structure is to be seen as a simple thickening of the somatopleure, similar in form to that described and figured in the youngest embryos of this stage (Fig. 17), for a region just back of somite VI. The region in which the duct is formed is throughout immediately ventral to the constriction separating the protovertebrae from the lateral plates.¹

¹ In sections from the posterior end of the embryo, it is necessary to guard against the false appearances which arise from the obliquity of the plane of the

The mode of development which I have described in the foregoing pages, taken in connection with frontal sections, which show that the pronephric thickening tapers gradually backwards into indifferent somatopleure, seems to me to be very strong evidence concerning the precise origin of the duct. I believe I am justified in concluding that *the segmental duct between somites V. and IX. arises in situ from a thickening of the somatopleure serially equivalent to that from which in the anterior region the pronephros is developed.* Indirect evidence which can be brought to bear on this question will be reserved for the fuller consideration which can be accorded it, in connection with the following stage (page 222).

STAGE IV.

**Plate I. Figs. 8, 9. Plate III. Figs. 18-26. Plate IV. Figs. 29, 30.
Plate V. Fig. 45.**

I have placed in this stage embryos of frogs taken from five different killings. They all belong to the fourth day after fertilization, and aside from individual variation show an evident advance in organization on the preceding stage. In all a distinct differentiation of muscular tissue has begun, the auditory vesicle is wholly cut off from the epidermis, and the ventral sucking (or more properly sticking) disks are well developed. In the following description, I shall find it convenient to distinguish a younger and an older set of embryos. In the younger set the embryos are from $3\frac{1}{4}$ to $3\frac{1}{2}$ mm. long; they have about 14 protovertebræ and the fundamentals of 3 pairs of gills. The embryos of the older set are from $3\frac{1}{2}$ to $3\frac{3}{4}$ mm. long; they possess about 17 protovertebræ and the fundamentals of 4 pairs of gills.

All the embryos of this stage have the pronephric pouch in its typical form. A side view of this organ with the neighboring portion of the section to the vertical axis of the protovertebra. Cross sections in this region frequently encounter two contiguous protovertebræ. If the plane of the section traverse the communicating canal of a protovertebra, it would also pass obliquely through the dorsal portion of the next anterior protovertebra. The latter would then appear in cross section as a distinct mass immediately lateral to the neural tube and the chorda, and would resemble the condition which a protovertebra presents when cut near its anterior or posterior wall. Immediately below this mass there would be found on the same cross section the ventral portion of the more posterior protovertebra, with the corresponding part of its cavity. The latter, however, being apparently a direct continuation of the body cavity, owing to the existence of the communicating canal, would appear to represent the dorsal part of the body cavity, and the fundament of the duct would thus seem to be farther removed from the dorsal angle of the body cavity than it really is.

segmental duct is shown in Figure 39 (Plate IV.). In this drawing, the outlines were obtained by reconstruction from a series of cross sections. The pronephric pouch is suspended from the dorsal angle of the body cavity by the nephrostomal funnels. Elsewhere it is wholly cut off from the mesoderm, and merely rests conformably on the outer surface of the somatopleure. The precise relations of the parts can be understood by referring to the series of cross sections shown in Figures 18 to 22 (Plate III.). Figure 18 represents a section through the left pronephros in the region of the first nephrostome. The location of the plane of this section in the reconstruction is indicated by the dotted line 18, in Figure 39. The lateral plates are here wholly cut off from the protovertebrae, splanchnopleure and somatopleure being continuous with each other at the dorsal angle of the body cavity. Figure 19 shows the structure of the organ between the first and second nephrostomes. In this and the following sections it was found advisable to depict the pronephric structures of the *right* side in order to exhibit in each case the section which most clearly showed the structural conditions. The next drawing (Fig. 20) in the series represents a section through the second nephrostome. In the preceding section,—not figured,—the three portions into which the lumen is here divided are continuous. The constriction between the middle and the ventral lumen is artificial; for the cells occasioning this local closure do not belong to the proper wall of the pouch, but form a group within the cavity. In several instances I have observed such groups of cells lying entirely free in the lumen of the pouch (Plate V. Fig. 45). In the present case, however, the mass is very intimately connected with the adjoining walls. This condition is preserved through a distance corresponding to the thickness of two or three sections, but the mass terminates by becoming free from both walls, so that in cross section it has the appearance of an "island" of tissue occupying the lumen of the pocket. The occurrence of these islands within the cavity of the pouch is of significance in determining precisely how the organ is developed. It is difficult to comprehend how they could be formed, provided the canals were produced by a fold of the somatopleure. On the other hand, they are perfectly intelligible on the assumption that the canals arise by the rearrangement of a solid mass of cells into a peripheral layer with a central lumen. According to the latter view, the islands would represent residual portions of the pronephric thickening which had not been transformed into peripheral wall.

Returning now to the section last under consideration (Fig. 20), the

ventral union of the walls of the pronephric cavity is, as I have shown, artificial; the constriction between the middle portion of the lumen and the dorsal, or nephrostomal, portion is more apparent than real, for it is formed by the posterior wall of the nephrostomal tube, the plane of the section not having cut exactly in the axis of the tubule. In the section following that shown in Figure 20, the pouch is detached from the peritoneum, and presents an appearance similar to that shown in Figure 19. Before the third nephrostome is reached, the canal is divided by a horizontal constriction into two tubes. The dorsal portion forms the tubule of the third nephrostome; the ventral portion is the anterior end of the segmental duct. Figure 21 shows these parts in the region of the third nephrostome. The section corresponds in position with the dotted line 21 in Figure 39.

In the following sections the duct rapidly assumes a more dorsal position (compare Fig. 39). It then proceeds directly backward, at the level of the constriction between protovertebræ and lateral plate. Figure 22 shows the duct in the region of somite VI. It has not yet been formed, however, throughout its entire length. On passing posteriorly, it gradually loses its lumen; then the circular arrangement of the nuclei indicating the position of the lumen also vanishes; the structure at length terminates as a simple thickening of somatopleure in the region of the tenth somite. In a few individuals, however, I found slight evidences of a mode of ending different from that just described. In one case the indications seemed so strong as to compel me to seek confirmation of the view that the duct takes its origin *in situ*. I shall therefore give the details of the evidence on this point, and discuss its probable significance.

Figure 23 represents in cross section the fundament of the duct in this specimen, as shown in the fifth section in front of its termination. The section of the mass here contains about eight cells, which are in close contact with the somatopleure. In the second section behind this one there are shown parts of four or five cells (Fig. 24). The protoplasmic patch in the centre (*cd.*) is wider than an average cell of the fundament, and probably represents the anterior ends of two cells lying in the following section (Fig. 25, *c.* and *d.*). Dorsal to this mass of protoplasm is a nucleated cell (*b.*), and above this a small area of protoplasm with a faint nucleus (*a.*) which is doubtless a portion of a cell the principal part of which was cut off by the preceding section. On the ventral side of the centre of the fundament there is also a round nucleated cell (*e.*). In the next following section (Fig. 25), there are two nucleated cells in the

centre of the mass (*c.* and *d.*), which, as I have said, doubtless correspond to the central protoplasmic area (*cd.*) seen in the preceding section. The most prominent cells of that section are here represented by two faint circles of protoplasm (*b.* and *e.*). In the next following section, not figured, the duct terminates as a single non-nucleated mass, probably corresponding to the dorsal cell in Figure 25. This remnant lies in a distinct depression of the somatopleure (Fig. 26, *f.*). This depression continues backwards through the space of three sections. Instead, then, of terminating in a thickening of the somatopleure, the end of the duct lies in a groove of unmodified somatopleure. There is no tissue directly behind the duct for its further growth, and the inference is natural that the somatopleure is mechanically depressed before the growing tip of the duct. In fact, I believe this to be actually the case, and that in this region the duct does grow by a simple cell proliferation within its own mass.

The key to the situation is to be found in the location of the posterior end of the duct in this specimen. An enumeration of the somites shows that the sections figured lie at the hinder end of somite XI. To show the bearing of this fact, I shall anticipate some of the results of a study of Stage V. In a series of frontal sections of the latter stage, I have succeeded in locating with reference to the successive somites the position at which the duct opens into the cloaca. The openings are in the same vertical plane with the middle of somite XII. The posterior end of the duct, then, in the specimen which I have just described, is within the distance of half a somite from its final termination. In order to empty into the cloaca, the duct has to grow inward from its position at the lateral margins of the protovertebrae to a position much nearer the median plane. It is difficult to comprehend how the duct could make this extension, except by proliferation of its own cells. It is just in this region that I find evidences of such a mode of growth. If the inference I have drawn from the facts adduced be correct, it seems to me to add strength to the conclusion I have reached in regard to the general mode of formation of the duct, inasmuch as it has been shown to be possible to detect free growth where it exists.

That the duct arises in the way I have described, and is not developed from the ectoderm, is shown, moreover, by certain indirect evidence which may be properly discussed at this point. As I have already stated, the duct is developed in such intimate connection with the somatopleure that I have been led to believe that it arises throughout its entire length from a proliferation *in situ* of that layer. In almost all of my prepa-

rations the duct in its backward growth is separated by a considerable space from the ectoderm, and I have observed no instance in which it was impossible to distinguish a perfectly sharp line between the fundament of the duct and the overlying ectoderm.

In describing the germ layers in Stage I., I referred to certain histological criteria which might be employed in determining to which germ layer a given group of cells belonged. The most valuable of these is the difference in the size and abundance of the yolk spherules, which even in that early stage served to contrast sharply the mesoderm from the ectoderm. In later stages, this character is equally pronounced. When the duct appears, the cells which constitute it are not distinguishable in histological features from those of the adjacent mesoderm, but are very different from those of the neighboring ectoderm. It seems to me extremely improbable that the cells of the fundament of the duct, with their numerous large yolk spherules, should have been recently derived from those of the ectoderm, which are provided with only few spherules of much smaller size. It would be entirely contrary to our conceptions of the physiological nature of yolk, if in the course of embryonic development this material was increased instead of diminished in quantity.

A similar argument seems to me to afford evidence that the duct arises *in situ*. If the duct had grown freely backward from an anterior proliferation, such growth would in all probability have been associated with the consumption of yolk in the cells of the fundament, and the spherules would be smaller or less numerous than those of the adjacent mesodermal cells. This, however, is not the case.

I conclude, therefore, that the segmental duct arises throughout its entire length by a proliferation *in situ* of the somatopleure. Its posterior end, however, grows across to the cloaca free from adjacent tissue.

Returning to the pronephric pouch, I purpose describing the relations of that organ to the somites. The section represented in Figure 29 (Plate IV.) shows graphically these relations. The plane of section in this case was very nearly tangential to the somatopleure at the points where the nephrostomes emerge. In this section it is evident that the three nephrostomes lie precisely under the first, second, and third somites, behind the ganglion nodosum. These correspond to the somites which I have numbered II., III., and IV.; so that the pronephric pouch remains in the same position as the pronephric thickening of earlier stages. In Figure 21 (Plate III.) the last remnant of the canal connecting the body cavity with the cavity of the protovertebræ is faintly indicated (above the letters *cœl.*) in the same transverse

plane as the third nephrostome; and Figure 6, as we have seen, shows more plainly the same condition in the case of the second nephrostome at an earlier stage.

The structure of the protovertebræ in this stage (Plate V. Fig. 45) merits especial consideration. Already in younger stages there is a differentiation of a peripheral epithelial layer surrounding the dense central mass, or kernel of the protovertebra. Laterally this peripheral part is represented by the entire somatic layer, which is separated from the kernel by the protovertebral cavity (*cœl.*). Along the median and ventral boundaries of the somite, a layer having an epithelial character is also to be seen. Thus the central mass which is to develop into the myotome lies on the median side of the coelom, and is wholly surrounded by an epithelial layer. Frontal sections show that this layer can be traced inward for some distance between successive somites, both from their median and lateral surfaces. Since the development of the protovertebræ proceeds from before backwards, a single frontal section shows successive stages in the changes which they undergo. From such a section it is apparent that neither the median nor the lateral portion of the peripheral layer develops muscular fibres. That portion of this layer, however, which is included between the kernels of successive protovertebræ, is apparently differentiated into muscle, and becomes merged in the myotomes. Very soon after the first development of muscle fibres in the myotomes, the peripheral portions which have not been converted into muscle separate from the central mass, and, while yet adhering in a lamella, show evident signs of disassociation. It is to be noted, that, in regions where traces of the communicating canal are still distinguishable, the median peripheral layer, not the kernel, is seen to be continuous with the splanchnopleure. The somatopleure, on the other hand, may be traced, as before, into the outer layer of the protovertebra. This peripheral layer I believe to be wholly converted, with the exception stated, into mesenchymatic tissue. In the stage before us we see that it is distinctly breaking away from the myotome, and that the cells are acquiring a flat tile-like form. In the following stage no layer that could properly be called epithelial is present. In its stead there is a considerable quantity of loose mesenchyme, and the lateral face of the myotome is covered by a sheath consisting of very delicate fibrillar connective tissue.

Not merely is mesenchyme produced by the thin peripheral layer of the protovertebræ, but in anterior regions considerable portions of the kernels of the protovertebræ also undergo a metamorphosis in this direc-

tion. Thus, if I be not mistaken, a protovertebra immediately in front of somite I. has been wholly converted into mesenchymatic tissue; the kernel of the succeeding protovertebra (somite I.) has given rise to a considerable quantity of mesenchyme; and the process has been manifested, though to a less degree, even in succeeding somites. Furthermore, having established the continuity of splanchnopleure and somatopleure with the median and lateral peripheral layers respectively of the protovertebræ, it seems to me the more probable that the former as well as the latter may give rise to mesenchyme. I have, in fact, seen conditions directly in front of the first nephrostome which indicated a very extensive production of mesenchyme from the lateral plate in that region.

My reason for dwelling at so great length on the derivatives of the peripheral layer of the protovertebra is, that this layer plays an important part in forming certain accessory portions of the pronephric system. I refer to the *capsule* of the pronephros. Already in the preceding stage I noted the occurrence of a lateral fold of the somatic layer immediately dorsal to the constriction between protovertebræ and lateral plates (Fig. 6). In the younger individuals of Stage IV. the fold covers the dorsal surface of the pronephric pouch, and extends a short distance down on its lateral surface (Figs. 18-21, *fund. cps.*). In the older set of embryos it has reached the somatopleure ventral to the pronephros, and thus forms a complete investing capsule.

In frontal sections the fundament of the capsule may be seen to consist of a series of segmental outgrowths from the successive protovertebræ. Later, these segmentally arranged structures fuse into a continuous enveloping sheet.

Lateral to the pronephros the capsule presents in general a two-layered condition, the result of its having been formed as a fold; but on ascending to the level of the lower boundary of the somite, these two layers separate (Plate V. Fig. 45); one passes beneath the protovertebra, covering the pronephros on its dorsal aspect; the other is continuous with the somatic layer of the protovertebra, forming a lateral sheath to the myotome. These layers are present in the region both of the pronephros and of the duct, but are seen in their simplest condition in the region of the second nephrostome (Fig. 20); not merely because this is the middle of the pronephros, but also because the process is somewhat modified in the protovertebra next in front of it (somite II.). Somite II. is one of those in which a considerable portion of the kernel of the protovertebra is converted into mesenchyme. For this reason the inner layer of the capsular

fold, after separating from the layer which forms the lateral sheath of the myotome, passes inward, and is there lost in a loose mass of tissue (Fig. 18), resulting from the disassociation of certain cells of the somite in that region. Intersegmental regions also present appearances which are confused by the occurrence of cells belonging to the partition between two successive somites. The points which I especially wish to emphasize in this description are (1) the origin of the capsule from the somatic layer of segmented mesoderm, and (2) the fact that the layer from which the capsule is developed is also in other regions converted into mesenchymatic tissue.

In the younger specimens of this stage a horizontal fold of the splanchnopleure is to be noticed, forming a slight ridge directly across the body cavity from the pronephros. It first appears in front of the second nephrostome, and develops from this point backwards. It is the fundament of the glomus or pronephric glomerulus.¹ In the earliest trace of this organ that I have been able to find (Plate I. Fig. 8) there were already a few small mesenchymatic cells (*ms'chy.*) located in the angle of the fold. The source of these cells I have been unable to determine with certainty. The nuclei of all the cells in the fold itself lie very close to the body cavity, and it does not seem probable that those small cells could be produced by delamination from the splanchnopleure without an actual migration of the nuclei of the somatopleural cells to the basal, or entodermal, surface of that layer. I have never seen signs of such migration, and I therefore do not believe that it occurs. Furthermore, the folded portion of the somatopleure does not at once become thinner than the neighboring portions of that layer. In older stages, such a thinning takes place, but it seems to be due to a superficial extension of the layer, rather than to delamination. The position of the nuclei of the large entodermal cells in this neighborhood is equally unfavorable for the formation of these small cells by delamination. The only remaining explanation is that the latter have migrated into their present position from relatively remote parts. Other loose cells may be found between entoderm and splanchnopleure, and the question here raised is only a part of the larger problem as to the source of all such cells, including those which bound the yolk veins. The fate of the cells which I have found in the fundament of the glomus, I shall consider in treating of a later stage. I may, however, here anticipate to the extent of stating that they are connective-tissue elements.

¹ The former term seems to me preferable, and will be employed in the following pages. The exact relations of the glomus to the mesonephric glomeruli will be explained in the general discussion.

Figure 9 shows the fundament of the glomus in one of the older embryos of this stage. Within the hollow of the fold may be seen two cells (*ms'chy.*), which are to be regarded as the descendants of the first small cells to which I referred in the younger embryo. Their differentiation in the direction of connective tissue can be noticed throughout the whole extent of the fundament. The scattered rounded cells near them probably represent embryonic blood cells in the region of the aorta.

STAGE V.

Plate I. Fig. 10. Plate IV. Figs. 31-34, 40.

The embryos belonging to this stage are on an average about thirty hours older than those of Stage IV. At this period almost all of the eggs are hatched; and, the duct having opened into the cloaca, the pronephros becomes functional. The larvæ of this stage measure 5-7 mm. in length, the rapid increase in size being largely due to the growth of the tail.

The form which the pronephros presents in this stage has been studied by means of reconstructions in the case of four pronephridia. The diagrams on Plate IV., Figures 31 to 38, represent in a rough way the number and distribution of the convolutions which the tabules present in this and the following stage. Of these, Figures 31 to 34 relate to the present stage. Figure 40 is a more accurate view of the pronephros which I have diagrammatically represented in Figure 32. In Figure 40 the outlines were taken with but little modification from the original reconstruction. I have not hesitated, however, even in this case, to remove defects plainly due to artificial causes, such as distorted sections and inaccurate superposition.

Comparing this drawing with Figure 39, it is easy to follow the changes that have taken place. In the earlier stage the fundaments of the three tubules are already present. The first modification which may be noted is the deepening of the constrictions which are indicated between the successive nephrostomes. In this way are formed three transverse tubules, joining distally a longitudinal canal; the former are the nephrostomal tubules, the latter I shall call the *collecting trunk*. In this case the continuation of the collecting trunk pursues a nearly straight course to the posterior margin of the gland, where it emerges as the segmental duct.¹ A second change which is apparent in Figure 40

¹ In Figure 40 the first nephrostomal tubule and the collecting trunk have a pink color, the second tubule is yellow, and the third is orange, whereas the segmental duct is uncolored.

is the growth of the collecting trunk in the region between the second and third nephrostomal tubules, and the consequent separation of the latter. The further complication in this case is mainly due to a convolution of the second tubule; slighter contortions occur in other parts. In the case of the pronephros diagrammatically represented in Figure 31, however, a canal, which corresponds to what we should regard in Figure 32 as the anterior portion of the segmental duct, has been folded first forwards, reaching nearly to the level of the first nephrostome, and then backwards. The bends which are convex anteriorly may be called the anterior bends; those which are convex posteriorly, the posterior bends. The universal occurrence of this condition in all older embryos makes it desirable to distinguish this bent portion of the tube and its derivatives both from the original longitudinal canal of the pronephros, which I have called the collecting trunk, and from the straight posterior portion, or segmental duct proper. In the following pages I shall speak of each *nephrostomal tubule* as extending from its origin in the nephrostome to its junction with the longitudinal canal, or collecting trunk. In the case of the first nephrostomal tubule, the point of union with the collecting trunk is usually marked by an abrupt change of direction; where this does not occur, however, the distinction between the two portions must be somewhat arbitrary. The *collecting trunk* forms the continuation of the first nephrostomal tubule, it receives in its backward course the second tubule, and may be regarded as terminating at the point of entrance of the third tubule. The *common trunk* arises from the point of junction of the third tubule with the collecting trunk, and, after making various convolutions, leaves the gland at its posterior end as the segmental duct. In the two pronephridia shown in Figures 31 and 33, we have before us examples respectively of the two principal forms of convolution which are to be recognized in subsequent stages, viz. the contortion of the second tubule and that of the common trunk. The third tubule finally undergoes convolution to some extent; but the first tubule and the collecting trunk take almost no part in the process. Although complication has appeared both in the second tubule and in the common trunk, it is to be noticed that these processes do not have a fixed sequence. I have numbered the diagrams on Plate IV. with reference to the state of development shown by the larvæ. In doing this, I have not been guided by the age alone, for the large amount of individual variation makes that method nearly valueless; but I have endeavored, by passing in review a large number of characters, to gain a notion of the relative degree of development shown by the larvæ.

The first of the series of diagrams (Fig. 31) shows complication to have taken place to a considerable extent in both the convoluted regions. In the next diagram (Fig. 32) the second tubule alone takes part in the complication. Figures 33 and 34 represent respectively the right and left pronephridia of one individual. In the right pronephros (Fig. 33) the typical condition of the common trunk is present, while the nephrostomal tubules have undergone no contortion. Likewise in the left pronephros (Fig. 34) it is the common trunk to which the increasing complication is due; but in this case there are two additional bends introduced by a slight folding backward of the middle of the anterior bend. The convolutions of the common trunk lie principally in the ventral portion of the gland. The tubes which in cross section are seen in the dorsal part are mainly the several nephrostomal tubules, and the collecting trunk. This condition is likewise retained in later stages.

The position of the pronephros with reference to the myotomes has not changed since the preceding stage. The whole structure is slightly longer, but the myotomes have also lengthened to the same extent. The three nephrostomes are situated, as before, beneath the first, second, and third myotomes posterior to the ganglion nodosum, and are segmental in position.

In all the embryos of this stage the duct has opened into the cloaca. It is to be remembered in this connection, that the morphological position of the duct is outside the somatopleure; so that the coelom and two layers of mesoderm intervene between it and the intestine. As might be expected, the union does not take place until the segmented and unsegmented portions of the mesoderm have become separated from each other. The passage to the cloaca is then effected through the split thus produced, and consequently around the dorsal angle of the body cavity.

In the frog, there is a sharp histological contrast between ectoderm and entoderm, and there is therefore no difficulty in assigning a limit to the proctodæal invagination. The region into which the duct opens is the hind gut, and the intestine at this point is unquestionably lined with entodermal cells. The portion of the primitive gut posterior to the openings of the segmental duct forms the Amphibian cloaca, and corresponds precisely, I should say, with that part of the cloaca of Amniota which Gadow ('88, p. 28) has recently designated by the name *urodeum*. The wall of the intestine is not wholly passive in the union occurring between it and the duct. In front of the excretory openings, the lumen of the intestine has an elliptical form, its major axis being vertical.

On passing backwards, the dorsal half broadens and finally exhibits two lateral processes, or cornua, the walls of which are composed of a layer one cell deep. The ducts open into the distal ends of these cornua (see Fig. 27, showing the condition in Stage VI.). Behind the outlets of the segmental ducts, the lumen of the intestine has a nearly circular outline, and descends rapidly to the anus, or, as it may now more correctly be called, the cloacal aperture. I was able to see in Stage IV. faint traces of these intestinal cornua. The cells of the dorsal roof of the intestine showed in this region a looser structure, and a line of pigment indicated the region of the outfolding. The cells of the duct and those of the cloaca are histologically very different from each other, so that it is for a long time possible to draw a line sharply separating the two constituents where they have come in contact.

The pronephric system of tubules presents in this stage quite uniform histological characters. I shall therefore describe its typical condition, and then consider the modifications that are to be found in certain of its regions. The walls of the tubules are very thick, measuring on the average about $25\ \mu$ in thickness. They accommodate themselves readily to the structures with which they come in contact, becoming thinner opposite elevations in neighboring surfaces, and thicker next to sinuses. The size of the lumen varies greatly. In the segmental duct proper, the diameter of the lumen is about $25\ \mu$; it is usually somewhat greater in the region of the convoluted tubules. The walls of the tubules are composed of an epithelium, consisting of a single layer of columnar cells. The radial dimension of the cells in the case of thick walls is approximately three times their width. Where the plane of the section cuts the wall of a tube tangentially, the cells may be seen to have a polygonal outline. The nuclei invariably occur close to the central lumen of the tube; each is large, and is usually provided with a single distinct nucleolus. The eccentric position of the nuclei is attended with a corresponding distribution of the cell protoplasm. By the picro-carmin method which I have employed, the yolk spherules take a bright yellow stain, and the nucleus a light red. The active protoplasm has a faint pink coloration, which, however, is wholly invisible if too much picric acid be left in the preparation. In young cells, where only a small amount of yolk has been consumed, the delicate tint of the protoplasm cannot be seen, since all the light passing through the section encounters yellow yolk spherules. As the consumption of the yolk progresses, the protoplasmic matrix comes into view. In the wall of the tubules, the yolk is crowded to the outer surface of the cell, and a sheet of protoplasm

first becomes visible close to the lumen. It is here also that pigment makes its appearance.

The histological character of special regions now claims our attention. The pronephridia shown in Figures 31 to 33 are all histologically very similar, but in the case of the gland represented in Figure 34 some notable differences occur, which I shall consider later. The somatopleure covering the pronephros is at this stage very thin. Each of the cells composing the membrane is thickest in its central portion, and tapers rapidly towards its margins. In the more advanced larvæ, the cells have elongated to such an extent that the peripheral portion is reduced to a thin protoplasmic plate, which is nearly devoid of yolk spherules. The central mass, on the other hand, contains the nucleus, and nearly all the yolk spherules. The peritoneum is continuous with the columnar epithelium of the walls of the tubules at the outer rim of the nephrostomes, which have the characteristic form of a funnel. Before reaching the periphery of the funnel, however, the columnar layer becomes slightly thinner, and at the rim it tapers rapidly, until it becomes continuous with the peritoneum (compare Fig. 18 of a younger and Fig. 28 of an older stage). The nephrostomal funnels are always deeply pigmented. The pigment is most abundant along the incurved surface, but is quite dense even up to the rim. It continues for a variable distance into the tubules. In the case of the pronephros, represented in Figure 31, the whole system of tubes was pigmented from the nephrostomes to the posterior bend near the beginning of the common trunk. The pigment granules are always disposed in a layer along the free surface of the cells. The nephrostomal tubules show in general the typical character, which I have previously described. The collecting trunk appears to be quite rigid, for I have never seen such a reduction of its lumen due to pressure as other tubes exhibit. The calibre of this canal is usually larger than that of the nephrostomal tubules. The portion of the duct which lies behind the third nephrostome is nearly straight and of uniform calibre. Generally the lumen is slightly wider, and the wall thinner, than in the nephrostomal tubules. In the pronephridia, shown in Figures 31 and 33, the loop embracing the anterior bend of the common trunk seems to have but little rigidity. It follows a tortuous course, and frequently the walls are so closely pressed together that the lumen is locally obliterated.

A peculiar modification in the pronephros, represented in Figure 34, has been alluded to. In this case, the common trunk, after proceeding from the level of the third nephrostome for a certain distance forward

along the ventral border of the gland, as in the other embryos, undergoes a change of structure at about the level of the second nephrostome. The lumen there begins to enlarge, and the wall to become thinner. Farther forward, the cavity of the tube becomes greatly dilated, and the bounding wall is reduced to a delicate pavement epithelium, having the same appearance as the peritoneum covering the pronephros. The tube again contracts shortly before attaining its most anterior bend. A similar dilation also occurs in the following stage, in the description of which I shall again refer to the chamber thus produced and suggest its possible function.

The pronephros at this stage is completely invested in a loosely fitting capsular membrane. The cells of which this envelope is composed have become very thin, so that they form a delicate sheet not more than $6\ \mu$ thick. The nuclei occur in slightly enlarged portions of the cells. They are rather small, and show a tendency to be flattened in the plane of the layer. At the lower outer angle of the myotome, the capsular membrane is continuous with the myotomal sheath, as in the earlier stage. The capsule covers the pronephros so loosely as to leave extensive spaces between the enveloping membrane and the tubules. These spaces, together with those between the convoluted tubes, form an extensive and complicated system of sinuses, which bound the pronephric tubules on every side (compare Fig. 28, belonging to the next older stage). Behind the last nephrostome, a considerable space intervenes between the wall of the capsule and that of the duct. There is thus formed a single continuous but irregular channel, which accompanies the duct throughout its entire course; it is also prolonged into the region of the gland as a large ventral sinus, which is triangular in cross section (compare the lower of the spaces marked *sn. snq.* in Fig. 28). This channel is the fundament of the posterior cardinal vein. The course of the vessel may be traced at this stage for a short distance behind the point where the ducts open into the cloaca. There are two veins connected with the anterior end of the pronephros. Of these the ventral one is the larger, and is continuous posteriorly with the cardinal vein. The dorsal vessel of the pronephros also unites with the cardinal vein by means of the spaces between the tubules. After passing forward and leaving the pronephros, the ventral vessel proceeds medianwards to empty into the sinus venosus, this vessel constituting the ductus Cuvieri. The dorsal vessel of the pronephros can be traced forward into the head. In the somite next in front of the first nephrostome, it lies between the ganglion nodosum and the myotome. It can be traced for some distance along the base of the

cranium, passing close to the median wall of the auditory vesicle and the ball of the eye.¹

There are two kinds of cells found within the capsule of the pronephros concerning which I have as yet said nothing. Those which are more numerous are scattered, of circular outline and of uniform size. Each has in general three or four large yolk spherules, and the nuclei are rather

¹ In order to ascertain, if possible, what vein of the adult this vessel represents, it will be necessary to describe here its condition in later stages. In the oldest embryos I have examined, 8.5 mm. long, the vein runs forward from the pronephros parallel to the aortic root and its continuation, the carotid artery. The vein is separated from the arterial trunk by the ganglia nodosum and faciale. Recalling the earlier position of the vein, it will be seen that it has been transferred from the median to the external side of the vagus nerve. In an intermediate stage, I have been able to see the nerve during its transit through the vein, thus confirming an observation of Kastschenko ('87, pp. 275, 276).

Following the vein farther forward, it is found to pass immediately ventral to the auditory vesicle, directly in front of which it sends a branch around the ganglion faciale to the side of the cranium. Slightly farther forward the vein divides, its branches passing around the more anterior of two ventral processes of the ganglion faciale. The two trunks thus formed separate. One enters the orbit, and can be traced to the anterior end of the optic bulb. The other passes below the eye, and pursues a nearly straight course to the anterior horn lip.

A description of the venous system of the adult frog has been given by Gruby ('42) and by Ecker ('64-81). The distribution of the veins which enter the dorsal portion of the pronephros corresponds most closely with the internal jugular of these authors. From the figures of Goette ('75), however, there can be no doubt that the vein I have described corresponds to the one which he calls the external jugular. I have been able to find a vein entering the sinus venosus directly which agrees accurately with the inferior jugular of Goette, but I have found none corresponding to the one he calls the internal jugular. It is stated by Goette (pp. 759, 760) that the vein named by him external jugular receives large branches from the maxillary and mandibular regions. This character would seem to connect it with the external jugular of Gruby and Ecker. According to Goette (p. 765), however, the external jugular of Gruby and Ecker is the same as his inferior jugular. I believe this statement to be true, and it seems possible that Goette, who confesses that his studies upon the veins did not extend beyond the first larval periods, may have erred in his account of the distribution of these rudimentary vessels.

Since the preceding description was written, a paper by Marshall and Bles ('90^b) has appeared, which adds another to the divergent accounts I have reviewed. The inferior jugular of Marshall and Bles corresponds closely with the vein I have alluded to under that name. The anterior cardinal vein is described by these authors ('90^b, p. 236) as "formed by the union behind the ear of a jugular vein returning blood from the brain and dorsal part of the head, and a facial vein which lies superficially along the side of the head ventral to both eye and ear." These vessels are described in tadpoles, measuring 9 mm. in length. My own observations on larvæ of nearly this size do not agree with this description.

smaller than in the cells of the tubules. Very similar elements abound in the fundaments of blood-vessels at this stage, and it is evident that the cells are embryonic blood corpuscles. The spaces in which they occur constitute a complicated system of communicating blood sinuses, and are continuous with the lumens of the vessels entering and leaving the pronephros.

The other class of cells to which I have referred are mesenchymatic. I have carefully studied these cells in the endeavor to ascertain their precise origin. A mode of reasoning similar to that employed in discussing the probable origin of the inner cells of the glomus leads to the conclusion that the mesenchymatic cells of the pronephros cannot have been given off from the walls of the tubules. As I have stated, the cells in these walls are very thick, and their nuclei lie close to the lumen of the tube. Under these circumstances, it is difficult to understand how any cells of the tubule should divide so as to give off from their basal surfaces cells as small as those in question. The usual process of cell division, if it took place parallel to the surface of the layer, would result in the production of a small cell on the side toward the lumen and a large outer segment. Such a large cell might, it is true, by repeated divisions, break up into numerous small cells, but for several reasons I do not believe this to have been the case. If such a delamination and subsequent cell division took place, it would naturally be a conspicuous process; but I have never observed any evidences of it. This method of origin would involve a considerable thinning of the tubes, which does not take place.

There remain two other possible sources for the mesenchymatic cells of the pronephros. They may have arisen from the tissue bounding the pronephros, viz. the capsular membrane and the adjacent somatopleure, or they may have come from remote regions. In judging between these possibilities, it is important to consider the sudden appearance of the cells and the small amount of differentiation they have undergone. It seems to me highly improbable that they should have already accomplished any extensive migrations. Under these circumstances, such positive evidence as I am able to adduce is the more convincing. In studying the youngest stages in which mesenchyme was present in the pronephros, this tissue was usually found near the somatopleure or the capsule, and frequently consisted of a row of cells closely applied to one of these layers. Occasionally I have seen a layer of mesenchymatic cells arranged along the somatopleure in a very definite manner, so that the nuclei of the two layers lay directly opposite each

other and the intercellular regions precisely corresponded. In such cases the evidence seems strongly in favor of delamination, but I have never seen a nucleus dividing in that direction. This negative evidence, however, should have little weight, since all cell divisions occupy a comparatively short time, and are also obscured by the numerous yolk spherules. The observations just recorded agree very well with the rapid thinning of the somatopleure to which I alluded in discussing the histology of the tubules. I conclude, then, that the mesenchymatic tissue of the pronephros arises from the adjacent somatopleure, and probably also to some extent from the capsular membrane.

The glomus (Plate I. Fig. 10) has attained in this stage nearly its final dimensions. The lateral plate having become wholly detached from the protovertebræ, the glomus has the appearance of being attached to the wall of the body cavity at its dorsal angle (compare Fig. 9, of a younger stage, and Fig. 47, of *Bufo*). There is some individual variation, but in general it may be stated that the ridge constituting the organ under consideration extends continuously from the first nephrostome backward to a position slightly behind the third. It appears in cross section (Fig. 10) obovate, being attached by the narrower end. In structure it is very compact, so that it is difficult to locate with precision cell boundaries in the dense interior mass. The investing portion consists of a single layer of cells, which is continuous with the peritoneum. These cells are large, and have the form of spheres flattened on their inner surfaces (compare Plate VI. Figs. 49 and 50, *la. pi'ton.*, which represent this layer in *Bufo*). They are slightly pigmented, and a distinct row of pigment granules can usually be seen close to the inner surface of the layer. These outer cells are evidently the representatives of the large cells of the splanchnopleure which was folded, at a previous stage (see page 227), to form the earliest fundament of the glomus (Fig. 9, *fund. glm.*). In certain favorable regions I have seen a thin structureless membrane lying directly within the outer cellular layer (compare Figs. 49, 50, *mb. ba.*). When any of the cells of that layer become detached, which frequently happens, this basement membrane usually remains in place, and gives a sharp outer contour to the glomus in that region. Besides the compact mass of large cells there occur within the organ one or two cells in each section (compare Figs. 49, 50, *en'th.*) which have an elongated form. They lie close to the basement membrane, with which their long axes are parallel. In sections each cell has a central swollen portion containing the nucleus, from which it tapers in both directions. I have not been able to trace the delicate lateral portions to their terminations, but I believe that

these cells form a complete endothelial lining, which follows closely the delicate basement membrane. They doubtless represent the loose cells alluded to in Stage IV. as occurring within the fundament of the glomus. Their origin I have already discussed in connection with that stage, where I showed that they were probably not derived from the outer layer of the glomus. Although the structure of the central mass of cells is, it must be admitted, somewhat obscure, I have found no evidences of the complication which Hoffmann ('86, p. 591) has recently maintained for it.

On the contrary, a comparison of many individual cells in this mass with the loose cells in the cavity of the aorta has made me confident that most of the cells contained in the glomus are embryonic blood corpuscles. It is possible, however, that others are derived from infolded portions of the outer layer of the glomus. They appear to have no representatives in early stages of the organ. In my opinion, then, the glomus is essentially a blood sinus, the wall of which projects into the body cavity, carrying before it the peritoneal layer.

The junction of the two aortic roots takes place very nearly opposite the first nephrostome (compare Plate IV. Fig. 28, *rx. ao.*). The aortic trunk thus formed (Fig. 10, *ao.*), since it occupies the space between the chorda and mesentery, passes close to the attachment of each glomus.

The precise relations of the aorta to the glomus are rather difficult to observe, since the former is peculiarly liable to injury in sectioning. The interior of the chorda at this stage consists of a frail vesicular tissue, whereas its outer sheath is tough, and resistant to cutting instruments. In sectioning, therefore, it collapses, and occasions serious distortion of the adjacent parts.

In the younger individuals of this stage, the cavity of the aorta did not seem to be sharply marked off from the root of the glomus; in several instances, indeed, I was able to observe a continuity between the endothelium of the aorta and that lining the glomus. In older individuals I have repeatedly noticed distinct branches from the aorta passing into the glomus (Plate I. Fig. 10, *va. sing.*). These observations were made, however, only on the most favorable sections, and I have been unable to ascertain the number or distribution of such branches. In both of the two most obvious cases, however, the vessel entered the hinder end of the glomus. Occasionally, the vessel to the glomus seems to be only a lateral branch given off from a vessel which can be traced between entoderm and splanchnopleure for some distance ventral to the glomus (Fig. 10, *va. sing.*, the lower of the two dotted lines).

I have spoken of the expanded dorsal portion of the body cavity, into which the nephrostomes open, and which contains the glomus. This portion of the body cavity constitutes the so-called pronephric chamber. It is not to be regarded as a closed cavity. Elsewhere the somatopleure and the splanchnopleure are closely applied to each other, but there is absolutely no fusion of these layers ventral to the pronephros.

STAGE VI.

Plate III. Fig. 27. Plate IV. Figs. 28, 30, 35-38, 41. Plate VI. Fig. 51.

The larvæ included in this stage are in general two or three days older than those of the preceding stage. They are about 8 mm. long from the anterior end to the tip of the tail. In this stage, the body no longer tapers gradually from the branchial region to the posterior end; but a definite line of separation is established between the trunk and tail regions. In the tail a distinct membranous fin has appeared, both along the dorsal and the ventral median lines. The horn lips can be seen surrounding the mouth, and the external gills project prominently on both sides of the body.

The pronephros of this stage has developed along lines foreshadowed in the preceding stage. The general form of the organ can best be understood by reference to the series of diagrams (Figs. 35-37) and the reconstruction (Fig. 41) given on Plate IV. As will be evident at once, the gland has reached a high degree of complexity, produced, however, by a continuation of the same process of complication which had begun in Stage V. Thus the first nephrostomal tubule¹ and the collecting trunk retain throughout a nearly unmodified condition; the third nephrostomal tubule usually becomes slightly complicated; the second exhibits the greatest number of convolutions. The common trunk, however, is the part which has been principally concerned in producing the increased complexity of the gland. It is to be noted that this contortion is not of a wholly indefinite nature; indeed, there is considerable uniformity in the pronephridia of different individuals of the same stage of development. In Figure 31, representing a pronephros of a larva in Stage V., it is to be seen that there are only two bends in the common trunk, which extends forward to the anterior end of the gland. From this simple condition the later complications may be derived by a few simple steps. In order to follow the changes it will be advisable to

¹ The same colors have been employed for corresponding parts in both Figures 40 and 41. Consult explanation of Figure 41.

distinguish: (1) posterior bend adjacent to the collecting trunk, (2) ascending arm, (3) anterior bend, and (4) the descending arm, which is continuous with the segmental duct. The simplest condition which I have found in Stage VI. is represented in Figure 35. This diagram relates to a larva of *R. sylvatica* Le C., and it is of interest to note its close similarity to Figure 38, which represents the pronephros of a larva of *R. pipiens* Schreb. (halecina) of this stage. These two pronephridia will be considered together. In both, the ascending arm of the common trunk makes either an S-shaped bend or a loop interpolated near the middle of its course; the transverse portion, or anterior bend, is thrown into one or two slight folds, and the descending arm shows two loops, one in the middle of the gland, and the other near its posterior end. The two remaining diagrams (Figs. 36, 37), though taken from different individuals, are alike in all essential particulars. The principal changes from the condition shown in the simpler pronephridia just described consist in the development of an additional loop in the course of the ascending limb, and of several slight folds in the transverse portion; the loops present in the younger individuals of this stage have persisted and become more extensive. In the case of the larva whose pronephros is represented in Figure 37, I made a comparative study of the pronephridia found on the two sides of the body. The comparison showed that a slight want of symmetry existed between the two sides. Occasionally the direction in which equivalent tubes were bent did not correspond. On the right side of the body (the one figured), for example, the hindermost loop of the descending arm was formed by an inward bend, while in the left pronephros the corresponding tube is bent outward. In the descending arm of the left pronephros a small loop occurs in addition to those present on the right, while one of the two loops occurring in the ascending portion of the right side is almost unrepresented on the left; thus, the right pronephros approximates in this respect the simpler organ represented in Figure 35. A more striking anomaly of the left pronephros consists in the occurrence of a slight bend of the collecting trunk between the junctions of the second and third nephrostomal tubules, so that the latter connects with an ascending portion of the collecting trunk. Finally, the third nephrostomal tubule of the left side joins the collecting trunk farther posteriorly than does the one on the right side. In general, however, it seems to me that the several pronephridia studied show a rather remarkable uniformity even in the details of the arrangement of their tubules.

The position of the pronephros with reference to the muscle plates is

the same in this stage as in the foregoing. The lateral plate is now wholly cut off from the myotomes; but a study of serial sections shows that each nephrostome lies beneath a myotome. These myotomes correspond to somites II., III., and IV.

The course of the duct in this stage is the same as in Stage V. The openings into the cloaca (Plate III. Fig. 27, *dt. sg.*) are now situated at the bottom of a depression in the dorsal wall of the cloaca (*clc.*). While the excretory products enter the main cloacal chamber by a single aperture, a glance at the histological characters of the short median unpaired trunk shows that it is lined with entodermal cells, and is therefore really a diverticulum of the roof of the cloaca. The ducts of the two sides, therefore, are not to be regarded as uniting into a common trunk, but as opening separately into a dorsal diverticulum of the cloaca.

The histological characters of the pronephric system have not undergone any great changes since the preceding stage. Figure 28 (Plate IV.) shows a cross section of the left pronephros of the larva, whose right pronephros is diagrammatically represented in Figure 37. The plane of the section passes through the first nephrostome, and the transition from the pavement cells of the peritoneum to the columnar epithelium of the tubules is clearly shown. This section also shows, besides the first nephrostomal tubule, the anterior ends of two loops which belong to the transverse portion of the common trunk. The walls of all the tubules are thinner than in the preceding stage, and since the nuclei remain of about the same size as heretofore, they now occupy a far larger proportion of the cell, and in the case of the thinnest-walled tubules are frequently almost in contact with both the outer and inner surfaces of the cell. The amount of yolk in the cells is considerably lessened, especially in those parts which exhibit the greatest number of convolutions. In some cells, a single large spherule is the sole remnant of the formerly abundant yolk. Pigment is present as scattered grains in the walls of all the tubules; it also shows a tendency, as in the previous stage, to accumulate along the free surfaces of the cells. The nephrostomes, however, are densely pigmented on the surface that is directed towards the body cavity and the lumen of the tubule. The duct posterior to the pronephros (Fig. 30) offers no features worthy of special mention. It is accompanied throughout by the cardinal vein (*vn. crd.*), on the median side of which the earliest fundaments of the mesonephric tubules are visible.

I have described a special enlarged region of the convoluted duct in a larva of Stage V. A similar condition is apparent on both sides of the

body in the case of the individual whose pronephros is represented in Figure 35. The dilated chamber (Plate VI. Fig. 51) is here formed by a great expansion of that portion of the ascending arm of the common trunk (*trn. com.*) which is adjacent to the collecting trunk (*trn. clg.*). A similar dilated chamber occurs in the pronephros represented in Figures 36 and 41; but in the latter case neither the dilation of the lumen nor the thinning of the wall is very pronounced. In both these cases the expanded chamber is present in portions of the tubular system which are exactly equivalent to each other. Under these circumstances, the expansion of the *descending* limb of the duct occurring in the pronephros of Stage V. (Fig. 34) seems quite anomalous. The dilated chamber is invariably, however, superficial in position, lying close to the capsular membrane. I have been unable to reach an entirely satisfactory opinion regarding its function. Since it is situated so near to the nephrostomes, it does not seem very well adapted to serve as a reservoir for the storage of fluids secreted by the gland, for by far the larger portion of the secreting surface is situated between it and the duct. However, the chamber doubtless receives whatever fluids are gathered by the nephrostomes or are secreted by the peritoneal tubules, and it is possible that the enlargement exists solely for this purpose. In following the duct from the dilated region towards its outlet, a greatly contracted portion is reached, and this may serve for the better retention of fluid contained in the chamber.

The capsule in these larvæ is not so well marked as in those of the preceding stage. Between the pronephric tubules and the ectoderm there has arisen a considerable quantity of mesenchyme, and the capsule now appears merely as the line along which this mesenchyme comes in contact with the pronephric tubes and blood sinuses.

In discussing the blood supply for the preceding stage, it seemed advisable to consider the vessels in older larvæ as well, and I shall therefore merely refer here to the account given in that connection.¹

¹ In all the larvæ of this stage which I have examined, I have observed a peculiar sac, of which I have been unable to find any mention in the literature. In the oldest larva of this stage it consists of a capacious sinus lying in the triangular area bounded by the myotomes, the somatic peritoneum, and the ectoderm. It extends backwards from the niveau of the third nephrostome for a length of two or three myotomes, and appears to be closed upon all sides. The sac lies in a mass of loose mesenchyme, but possesses firm walls, so that any opening would naturally be easily recognizable. In the interior of the sac, cells which are undistinguishable from blood corpuscles are found in considerable numbers. In a younger larva the sac occurs in a corresponding position, is nearly filled with blood cells, and is in open

The glomus is somewhat larger and more compact than in the preceding stage, and for that reason its structure is more obscure; but I have seen nothing which would lead me to believe that it differs materially from the condition exhibited by the younger glomi of Stage V. The organ is bounded by a definite peritoneal layer and contains blood cells together with embryonic connective-tissue stroma. The blood cells are usually contained in definite channels, and, being closely packed together, frequently appear in cross sections to be disposed with considerable regularity around a central point. This arrangement is naturally suggestive of a tubular or a rod-like structure; but the histological characters of the cells and the conditions exhibited by adjacent sections show that this impression is illusory. In short, I have been unable to find within the glomus any traces of the rods and thick-walled tubes which have been described by Hoffmann ('86, p. 591).

No closed pronephric chamber exists at this stage. In the most anterior sections in which the pronephric tubules appear, a blind anterior diverticulum of the body cavity is to be seen; but this unites with the general body cavity surrounding the intestine even before the niveau of the first nephrostome is passed. Throughout the remainder of the pronephric region the lung bud (Plate IV, Fig. 28, *fund. pul.*) forms a ridge on the splanchnic side of the coelom. This ridge partially separates the pronephric chamber from the general body cavity; and in the region of the third nephrostome a still more perfect closure is effected on the right side of the body by means of the approximation of a portion of the midgut to the peritoneum covering the pronephros.

STAGE VII.

The age of the larvæ of this stage, reckoned from the time of fertilization, is about forty-seven days. A large gap therefore intervenes between Stages VI. and VII., and the older larvæ are studied merely for the purpose of observing the process of degeneration in the pronephros. In the larvæ of Stage VII. the mesonephros has already attained a degree of complication comparable to that gained by the pronephros at Stage VI., i. e. the same average number of tubes appear in cross sections through the two glands. The mass of contorted tubules in the case of the mesonephros, however, is formed wholly by the transverse tubules, while the communication with the cardinal vein. In a larva of intermediate age, the sinus communicates with the cardinal vein by means of a very narrow canal. Respecting the fate and the significance of this singular structure, I have no suggestions to offer.

duct pursues a direct course through the gland. The duct is situated in the dorsal portion of the mesonephros adjacent to the lower borders of the myotomes; its relations are therefore different from those of the longitudinal canal of the pronephros, since, as we have seen, the common trunk in the pronephros is greatly convoluted, and its windings occupy the ventral portions of the gland.

The marked signs of degeneration which the pronephros presents in this stage prevented my reconstructing the gland, since it proved to be impossible to follow any given tube throughout the entire series of sections. Indeed, I am convinced that the tubules are no longer strictly continuous. I must therefore content myself with a brief description of the histological features noticed.

The lumen of the tubules is greatly enlarged, and is frequently filled with a dense coagulum which stains similarly to protoplasm. The cell walls are very thin and show a tendency to become shredded or frayed along the interior surface. The membranes between the cells in the wall have become indistinct, and the number of nuclei in a given area is far less than in a corresponding portion of the wall in Stage VI. The nuclei are stained only feebly, but contain deeply staining granules, and seem to be disappearing, since one can observe numerous gradations between the typical nuclei and those which have become so pale as to be nearly invisible. The ground substance of the walls is slightly vacuolated and contains numerous scattered dark granules. Between this remnant of the cellular wall of the tube and the basement membrane, I have frequently seen small cells with deeply stained nuclei. These may possibly represent intrusive connective-tissue elements.

I regret that I have not been able to make an extended study of the degeneration of the pronephros; but the limit which I have set to my work is perhaps the least arbitrary which I could easily make.

B. Bufo.

The development of the pronephros and the segmental duct in *Bufo* is very similar to that which I have described for *Rana*. For this reason, I can treat the development in *Bufo* much more briefly, and shall lay principal stress upon those features which are unlike in the two genera.

STAGE I.

In the case of *Rana*, this stage included embryos which showed an ill defined somatopleural thickening lying immediately posterior to the cranial ganglionic mass. This proliferation proved, on comparison with

older specimens, to be the first indication of the pronephric thickening. A similar condition of the somatopleure is presented by embryos of *Bufo* about 2 mm. long, in which the medullary folds are widely open.

The general relations of the germinal layers at this stage are almost identical with those in *Rana*, and the same histological criteria for distinguishing them can be employed. The ectoderm is very sharply marked off from the mesoderm. The former is deeply pigmented, while the adjacent mesoderm is almost destitute of pigment. The yolk spherules of the ectoderm measure on the average about $2\ \mu$; those of the mesoderm, about $4\ \mu$.

In embryos in which the medullary tube is still widely open, the somatopleure and splanchnopleure are separated from each other by a distinct space, the coelom, which can be traced with perfect distinctness into the protovertebral plate, where it becomes slightly expanded. In the anterior half of the embryo, both the somatic and the splanchnic layers are only one cell in thickness. Posteriorly, and in the middle trunk region, however, certain loose cells bordering on the coelom become associated with the somatic layer; but this layer is never, except at the extreme hinder end, more than two cells in thickness.

STAGE II.

Embryos in which the medullary tube is just closed exhibit a condition of the mesoderm slightly different from that of Stage I. In the posterior portion of the embryo, the mesoderm is quite thick in the region of the protovertebral plate, and becomes gradually thinner as it approaches the ventral portion of the body.

Anteriorly, the protovertebral plate shows traces of the differentiation of four or five protovertebræ. Of these, the most anterior lies in the same transverse plane as the ganglion nodosum, and, following the method of designation which was employed in the case of *Rana*, would properly represent somite I. This protovertebra, as in *Rana*, shows signs of transformation into mesenchyme, and is considerably compressed in the region of the ganglion.

The thickening has the general form which I have described for the corresponding stage of *Rana*, and its anterior margin is situated under somite II.

In the region of its greatest thickness, which is somewhat lateral to the boundary between the protovertebra and the lateral plate, it is two or three cells deep. It thins out slowly on the ventral side, much more rapidly on the side of the protovertebra, or dorsally. The thickening

involves the ventral portion of the lateral wall of the protovertebra itself, although the greater part of the thickening is in the region of the lateral plate. I have not been able to find any sharp plane of division marking the lower limit of the thickening. The latter extends posteriorly through a distance of three or four somites, but it is difficult to make out its relations to the protovertebrae, in consequence of the small amount of differentiation which these exhibit at this stage. It seems to me, however, that the thickening reaches backward into a region posterior to that in which the pronephric tubules later develop, and therefore represents already the first fundament of both the pronephros and the anterior end of the segmental duct.

Frontal sections show the same relations between the pronephric thickening and the protovertebrae that I have described for *Rana*, but in *Bufo* the coelom is entirely obliterated by the growth of the pronephric thickening, and consequently the pronephric chamber described in a corresponding stage of *Rana* does not exist in *Bufo*. This circumstance renders the determination of the precise boundaries between the two layers of mesoderm somewhat more difficult in the Toad than in the Frog, but still there is usually an unmistakable line of division between somatopleure and splanchnopleure even in the former. The pronephric thickening at this stage is from two to three cells thick, and is a solid mass.

STAGE III.

In embryos of this stage, the fundament of a single pair of gill-folds is present; the fundament of the auditory vesicle consists of a simple thickening, which is just beginning to separate from the superficial ectoderm; and five or six protovertebrae have made their appearance. The embryos measure from 2.25 to 2.50 mm. in length.

The pronephric thickening becomes sharply marked off in this stage from the undifferentiated mesoderm lying ventral to it, and the canalization of the structure is accomplished by the arrangement of the cells around a lumen. Segmentally, the pronephric thickening has in general the form of a close fold of somatopleure, whereas intersegmentally it appears as a flattened tube. The points of continuity with the coelom are situated each directly beneath the middle of a protovertebra, and the somites in which they appear are II., III., and IV.

The duct arises as a backward continuation of the pronephric thickening, and contrasts very sharply in histological characters with the ectoderm, in consequence of the pigmentation and paucity of yolk spherules in the latter.

STAGE IV.

Plate V. Fig. 43.

Embryos of this stage measure from 2.8 to 3.1 mm. in total length. Muscular fibres have begun to appear in the myotomes, the auditory vesicles are entirely detached from the external ectoderm, and the protovertebræ have been differentiated as far back as the anus.

The pronephric pouch of *Bufo* is very similar to that of *Rana*. It communicates with the cœlom by means of three nephrostomes, and from its ventral margin the duct takes its origin. The nephrostomes are segmental in position, and are situated beneath protovertebræ II., III., and IV.¹

The duct can be followed for some distance posterior to the hindermost pronephric nephrostome as a distinct elliptical tube with a central lumen. The lumen, however, disappears further posteriorly, and the duct terminates either as a simple thickening of the somatopleure, or its posterior end merely rests upon the mesoderm in the region of somite XI. The hinder tip of the duct (Fig. 43, *fund. dt. sg.*) in both cases resembles very closely the adjacent mesoderm both in the size and in the abundance of yolk spherules, and it differs from the ectoderm both in these features and in the scarcity of pigment. In *Bufo* I have never been so fortunate as to find the growing end of the duct situated in a groove of depressed mesoderm; but I believe that the fundament extends itself from the region of its origin in the somatopleure to the projecting cornu of the cloaca by means of an independent growth on the part of its own cells. The greater part of the duct, however, arises from a local proliferation of somatopleure.

The pronephric capsule in *Bufo* arises as a downgrowth from the outer peripheral layer of the protovertebræ. In this stage, however, it has not reached the somatopleure ventral to the pronephros, but merely forms a two-layered scale-like sheet of tissue covering the dorsal portion of the gland.

The pronephric chamber is present at this stage. The general body cavity, however, has not yet appeared, the somatopleure and splanchnopleure being in other regions in close contact.

¹ I have preserved in the enumeration of the body somites of *Bufo* the same designations that were employed in the case of *Rana*. In *Bufo*, however, the kernel of the degenerate protovertebra in front of somite I. gives rise to a few muscle fibres

STAGE V.

Plate V. Figs. 42, 46. Plate VI. Figs. 47, 49, 52.

At this stage the larvæ were hatched and swam about freely in the aquaria. The larvæ measured from 4 to 6 mm. in length, and each had a distinct tail, which protruded for a distance of 1.5 to 2 mm. behind the anus. The pronephros was probably already functional.

The character of the convolutions of the pronephric tubules was studied in the case of four pronephridia. In this feature one of them corresponded very closely with the condition in the pronephros of *Rana* represented in Figure 33. The remaining pronephridia differed from this type solely in the circumstance that the third nephrostomal tubule joined the collecting trunk at the extreme posterior portion of the bend, which in *Rana* usually forms the first portion of the common trunk.

The position of the pronephros with reference to the somites remains in general nearly the same as in the preceding stage. In individual cases, however, the nephrostomes do not appear to lie precisely under the middle of the myotome.

In embryos of this stage, the segmental ducts already open into the cloaca. These openings are situated beneath myotome XII. It is obvious from this fact that the duct in the older embryos of Stage IV. had already very nearly reached the region of its final communication with the cloaca. In *Bufo* the lumen of the gut is very narrow, and is separated from the lateral walls of the body by an extensive mass of yolk cells. The cloacal cornua are therefore in this case very long, extending to the outer surface of the entoderm. The ducts reach these cornua by passing between the dorsal angle of the body cavity and the overlying myotomes.

The histology of the pronephros in *Bufo* does not present any noteworthy features of difference from that in *Rana*. The tubes are all slightly smaller in *Bufo*, and their walls contain somewhat more pigment than do those of *Rana*.

The capsule envelops the pronephros and duct in the way that I have described for *Rana*, and it also encloses a series of blood sinuses which are developed from the posterior cardinal vein. I was not able to obtain in *Bufo* any additional evidence in regard to the origin of the mesenchyme of the pronephros.

Two veins emerge from the anterior end of the pronephros. One of these is the immediate continuation of the posterior cardinal vein, which, in passing forward as the ductus Cuvieri (Plate V. Fig. 42, *dt. Cuv.*), makes a rapid ventral descent to open into the sinus venosus. The

other vein (Fig. 42, *vn. jgl.*) passes forward between the myotome and the vagus nerve. It evidently is one of the jugular veins, but I have not been able to study its distribution in later stages, and am therefore unable to state more precisely which vein of the adult it represents.

The structure of the glomus in *Bufo* is far more evident than in corresponding stages of *Rana*. In treating of the development of the glomus in the latter, I reached the conclusion that it arises as a simple fold of splanchnopleure, into which mesenchymatic cells migrate. In later stages I was able to identify the original outer sheath with a distinct basement membrane, and found within this membrane a large number of embryonic blood corpuscles, and occasionally certain cells which resembled in their histological characters those of the sheath or peritoneal layer. In *Bufo* the vascular system is less developed than in the corresponding stage of *Rana*; and, owing to the small number of the blood corpuscles, the remaining cellular elements come more plainly into view. The usual form of the glomus is that of a hollow peritoneal sac lined with endothelium (Plate VI. Figs. 47, 49, 50), and containing scattered blood corpuscles (Fig. 46). At the entrance to the sac the endothelium (*en'th.*) is continuous with the loose mesenchyme surrounding the aorta, and, in certain regions, the lumen of the latter can be traced into the interior of the glomus. This organ, then, exhibits markedly the character of a blood sinus, the walls of which project into the body cavity. Occasionally one encounters in *Bufo* certain minor pocketings of the peritoneal layer of the glomus, — invaginations into the lumen of the glomus at the place, e. g., occupied by the letters *cal.*" (Fig. 52). If the cells at the apices of such invaginations were to become detached, this condition would serve to indicate the source of the pigmented cells found in the interior of the glomus in the case of *Rana*, although I have as yet reached no final conclusion in regard to this matter.

In this stage the body cavity exists as a distinct lumen only in the region from which the nephrostomes emerge, where it constitutes a pronephric chamber.

My studies on the development of the excretory organs in *Bufo* have not extended beyond the present stage.

C. Amblystoma.

Plate V. Fig. 44. Plate VI. Fig. 48. Plate VII. Figs. 53-56.
Plate VIII. Figs. 57-65.

Amblystoma shows in the development of its excretory system many features of similarity to the Anuran forms already described. The dif-

ferences, however, are far greater than those which exist between *Rana* and *Bufo*, and will require for their presentation a fuller treatment than was given in the case of the latter genus; but the development in all three genera is sufficiently similar to allow the recognition of the same successive stages, based upon the degree of complication exhibited by the pronephros.

STAGE I.

Plate VI. Fig. 48.

In embryos of this stage, the two lateral medullary folds have just fused to form the neural tube. The embryos have a slightly elongated form and measure about 3.7 mm. in length. They are slightly more advanced than the embryo of *Amblystoma* represented by Bambeke ('80, Planche XI. Fig. 35). The eggs from which I derived my series of embryos had been deposited for a variable length of time before they were collected, and I am unable to give the ages of the several stages.¹

The general arrangement of the germ layers (Plate VI. Fig. 48) is similar to that which I have described for *Rana* and *Bufo*. The ectoderm (*ec'drm.*) consists in general of a single layer of cells, each of which has the form of a cube slightly flattened. Scattered ectodermal cells form an incomplete deep layer, which may gain in some regions, e. g. in the head, a very considerable development. The outer face of each ectodermal cell possesses a thin layer of pigment, but this is by no means so dense as in *Rana* and *Bufo*. At this stage yolk spherules are abundant in all the cells of the ectoderm.

The entoderm has nearly the same arrangement as in *Rana*, but the yolk cells are relatively more abundant, and the lumen of the gut is narrower. In the anterior region, the chorda consists of a simple fold in the dorsal roof of the intestine; but in the posterior portion of the body it is represented by a single row of high columnar cells, which form a layer convex from side to side towards the lumen of the intestine. This layer is the one which O. Hertwig ('83) has named the chorda-entoblast. The cells of the yolk entoderm are in general the largest in the

¹ A quantity of the eggs of *Amblystoma punctatum* Linn. raised in the laboratory during the present season reached the several stages as follows: Stage I., 5 days; Stage II., 5 days, 12 hours; Stage III., 6 days, 15 hours; Stage IV., 7 days, 15 hours; Stage V., 8-14 days; Stage VI., 15-20 days. These figures are only approximate, and between Stages II. and V. the individual variation is frequently more than sufficient to cover the entire interval between two successive stages. The temperature of the water varied somewhat during the period, but I believe that 10 or 11° C. would be a fair average.

body, and contain very large yolk spherules. The majority of the entodermal cells contain no conspicuous accumulations of pigment; but the latter may occasionally be found in considerable quantity, particularly in the cells bordering on the gut.

In the dorsal portion of the body, the mesoderm consists of two lateral masses of tissue, each of which spreads outward and ventralward from the neural tube, and joins its fellow of the opposite side in the ventral median line. Each of these masses of mesoderm is thickest next to the medullary tube, and gradually becomes thinner in passing outward around the mass of yolk cells. In the dorsal half of the body (Fig. 48) each mass of cells consists of two distinct layers, which are continuous with each other along the sides of the neural tube. They represent the first division into somatic (*la. so.*) and splanchnic (*la. spl.*) mesoderm, and the slight space which separates them is the cœlom (*cœl.*). On passing outward and ventrally, the two layers of mesoderm gradually approach, and at length are continuous with, each other; for a short distance farther, it is still possible to trace two rows of nuclei, indicating approximately the territory occupied by the layers; but this arrangement finally disappears, and before the ventral surface is reached the mesoderm has the form of a layer only one cell in thickness (*ms'drm.*).

In both somatic and splanchnic layers, the cells are of a nearly cubical form, but those of the parietal layer are rather thicker, and may be even columnar. The mesoderm of the ventral side of the body, on the other hand, is composed of more flattened elements. The cells of the mesoderm are in general intermediate in size between those of the ectoderm and of the entoderm. Their yolk spherules are much smaller than those in the entoderm, but resemble those in the ectoderm too closely to afford a thoroughly satisfactory criterion for distinguishing the two layers. The mesodermal yolk spherules are, however, *slightly* larger than those of the ectoderm; and in doubtful cases they may be taken into account.

The pigment of the mesoderm is usually collected along that surface of the cell which faces the cœlom, and may in part serve as a guide for following that cavity in cases where the bounding layers of mesoderm are in close contact with each other.

I have spoken of the somatic mesoderm as a layer a single cell in thickness; this is not, however, an adequate representation of the actual condition. In many sections there may be observed, from place to place, an additional cell associated with the otherwise single layer. The occurrence of an incomplete second layer of cells is most noticeable in the anterior portion of the trunk, in a region directly lateral to the protover-

tebral plate. It is probable that this slightly thickened somatic layer is the first indication of the pronephric thickening.

STAGE II.

Plate V. Fig. 44.

Embryos of this stage measure nearly 4 mm. in length; the medullary tube has become entirely separated from the superficial ectoderm, and three protovertebræ can be distinguished in longitudinal sections.

The fundament of the pronephros forms in this stage (Plate V. Fig. 44) an evident thickening of the somatic mesoderm lying immediately lateral to the protovertebral plate. Throughout the greater part of the thickening, the layer is obviously two cells thick, and occasionally three nuclei may be seen in a line perpendicular to its surface. The cells constituting the thickening are closely compacted, and do not appear to form definite layers. The fact that the thickening passes through a stage in which it is only two cells in thickness precludes the possibility of its being a disguised fold with closely applied walls, for in that event there must be at least three layers of cells involved. Neither the anterior nor the posterior limit of the thickening can be clearly determined at this stage. I am also unable to state definitely its relations to the protovertebræ, inasmuch as these cannot be adequately made out in transverse sections, and the extent of the thickening cannot be satisfactorily observed in such longitudinal sections as pass through both the protovertebræ and the pronephric thickening. The latter may be traced for a distance of about 0.5 or 0.6 mm. Each protovertebra at this stage measures about 0.27 mm. in length, so that the thickening extends through a length of about two protovertebræ.

In slightly older embryos the pronephric thickening becomes in general three cells in thickness; but it is still a solid proliferation, with no indication of extensions of the cœlom between the layers.

STAGE III.

Plate VII. Figs. 55, 56.

At this stage the young *Amblystomas* are about 4.3 mm. long and distinctly elongated in shape; but they show as yet no trace of a tail. They are further characterized by the possession of about eight well marked protovertebræ.

In all the embryos of this stage the pronephric thickening is at least three cells in depth, and has a definite ventral boundary. The thickening extends as far forward as the front face of somite III., and posteriorly

tapers gradually into undifferentiated somatopleure. The backward prolongation of the thickening is the first fundament of the segmental duct, and may be traced at least as far back as somite VI. Both portions of the thickening appear to arise in the same way; namely, by cell proliferation in the somatopleure.

It is a matter of some difficulty to ascertain when the first trace of a lumen appears. Before the two walls actually separate, the nuclei frequently show an arrangement which is suggestive of an evagination; but one cannot always trust such appearances. Later, a line of pigment can be traced from the body cavity for some distance into the interior of the thickening, and finally the two walls separate, leaving a clearly defined lumen. In all cases, the two regions of continuity with the cœlom are opposite the middle of protovertebræ III. and IV. respectively; and there is no indication whatever of a continuous fold.

Although the pronephric mass thus shows evident signs of segmentation, yet, as is to be seen by a comparison of segmental and intersegmental regions (Plate VII. Figs. 55 and 56), the proliferation is not interrupted in the latter regions. In frontal sections through pronephridia in which a definite lumen has begun to appear (compare Plate VII. Fig. 55), there can be seen two narrow canals leading from the cavities of protovertebræ III. and IV. and extending outward as cœlomic diverticula into the pronephric mass. From this condition the hasty conclusion might be drawn that the narrow canals are in fact outgrowths from the *protovertebral* cavities. This however, in my opinion, is not the case. If the relations of the mesoderm in such a transverse section as is shown in Figure 55 be regarded, it will be seen that a frontal section through the pronephric region (in the figure cited, a horizontal section a little below the level of the letters *col.*) would cut through the protovertebral cavity near its floor, and at the same time pass through the lumen of the pronephric thickening. Since, moreover, these two spaces are continuous by means of the communicating canal, it might at first appear that the latter belonged to the pronephric tubule. The fate of that portion of the tube, however, shows this interpretation to be incorrect, and that it was only by means of the communicating canal that the lumen of the pronephros communicated with the protovertebral cavity; for when the separation of the protovertebræ from the lateral plate takes place, the communicating canal, which is assumed to be the stalk of the pronephric diverticulum of the protovertebra, becomes closed, and the pronephros is thereby left in communication with the body cavity alone (compare Mollier, '90, Taf. XII. Figs. 10 c., 10 d., tr_1 and tr_2).

STAGE IV.

Larvæ of *Amblystoma* do not possess a conspicuous widely open pronephric pouch, such as has been described in Anuran species; but the proliferation becomes at once converted into a tubular organ. Indeed, the condition of the pronephric thickening in Stage III. is the one which is most similar to the Anuran pronephric pouch, since it is then a continuous structure having connections with the coelom in segmental regions.

In slightly older embryos, the dorsal half of the pronephric thickening is no longer continuous through the region between protovertebræ III. and IV.; and from this region backward to the hinder face of protovertebra IV. the mass is distinctly divided into two tubes. Of these two tubes, the more median and dorsal is the second nephrostomal tubule; the more lateral and ventral is the common trunk. Finally, it is to be observed in a number of cases that an anterior loop of the common trunk occurs a short distance in front of the point of junction with the nephrostomal canals. The pronephros thus has a form which approximates very closely to the condition which forms the starting point for the next stage (Plate VIII. Fig. 58).

STAGE V.

Plate VIII. Figs. 57-60.

This stage includes embryos which have attained a length of from 5 to 6 mm. Many of the older embryos of the stage have already hatched; they possess well developed tails and swim about freely.

The general form of the pronephros has been studied by means of a number of rough reconstructions, some of which are represented by the diagrams on Plate VIII. In Figures 57 to 60 inclusive, which belong to this stage, no windings have been reproduced which were not of sufficient magnitude to form definite antero-posterior loops; and, further, in plotting these loops, no attempt has been made to preserve in the diagram the natural direction in which the tube is actually bent. However, the relative positions of the bends in an antero-posterior direction have been accurately reproduced.

In the younger individuals of this stage, the pronephros (Fig. 58) resembles in many respects that of *Rana* represented in Figure 33; but it differs from the latter, notably in the occurrence of two instead of three nephrostomes and nephrostomal canals. For this reason, there is no canal which corresponds to the collecting trunk of *Anura*, save that

portion of the latter which intervenes between nephrostomes I. and II.; and in discussing the topographical relations of the tubules it will be needless to distinguish this remnant of the collecting trunk from the first nephrostomal tubule. In this simplest condition of the pronephros, the common trunk makes a single loop, the anterior curve of which is situated nearly as far forward as the level of the first nephrostome. In somewhat older pronephridia (Figs. 59, 60) the main bend of the common trunk occupies a position even in front of the first nephrostome, and a number of minor folds intervene between the junction of the nephrostomal canals and this most anterior fold. In none of the pronephridia of this stage is there any evidence of convolution in the nephrostomal canals.

One individual of this stage departed from the normal condition, in that it possessed three instead of two nephrostomal canals. This abnormality occurred on both sides of the body, and appears to be correlated with a less highly developed first nephrostomal tube. It is to be noted that the third tubule (Fig. 57) appears as an appendage attached to the most posterior loop of the common trunk. This topographical relation suggests that it is the most posterior of the three nephrostomal tubules which has been added to those normally present in *Amblystoma*, and this inference is shown to be correct by the relations which the several tubules bear to the body somites. The question whether the most posterior of the three tubules in this case represents the third nephrostomal tubule of the *Anuran* pronephros can be answered only by a consideration of the relations which the several nephrostomes in the two groups bear to the overlying protovertebræ, and will be recurred to in the general discussion which follows. I may here anticipate to the extent of stating that the first and second tubules of *Amblystoma* probably correspond respectively to the second and third of *Rana* and *Bufo*, the abnormal third tubule belonging to a yet more posterior metamere.

The position of the pronephric nephrostomes with reference to the myotomes was determined at an early stage by the location of the first metameric diverticula which are developed within the pronephric mass; and in the present stage these relations have not materially changed. The two nephrostomes of the normal pronephros lie beneath the third and fourth myotomes respectively. In the case of the pronephridia with a supernumerary nephrostome, the first two nephrostomes occur beneath myotomes III. and IV. respectively, while the third nephrostome is found beneath myotome V.¹

¹ Myotome I. of this enumeration reaches forward to the root of the vagus nerve, and is flanked on its outer face by a portion of the ganglion nodosum, exactly as in the case of the *Anura* described.

In this stage the segmental duct in the younger embryos shows somewhat different conditions from those found in the older embryos. In the case of unhatched embryos possessing the simple pronephros shown in Figure 58, the duct on passing backwards gradually diminishes in calibre, and finally loses all trace of a lumen. The fundament of the duct is in this region composed of four or five cells in each cross section, which are frequently arranged with some regularity about the centre as an axis. On proceeding to more posterior regions the fundament of the duct becomes intimately connected with the mesoderm, and is finally lost in that layer. In *Amblystoma* the histological characters of the mesoderm and the ectoderm are not sufficiently unlike to allow one to base on them a definite conclusion respecting the layer which has furnished the material for the fundament of the duct. In all cases which I have observed, however, the duct neither unites with the ectoderm nor terminates freely; but its posterior end invariably is closely applied to the mesoderm, and consequently is most probably derived from that layer. In view of the fact that the yolk spherules of the fundament of the duct are of the same size as those present in the adjacent mesoderm, I am of opinion that the duct has undergone no extensive independent growth, but has arisen *in situ* as a proliferation of the somatopleure.

In the older embryos of this stage, the duct has extended backwards to the region of the cloaca, and joins the latter near the posterior face of myotome XX. A distinct post-anal gut is present at this stage. Its anterior portion contains an evident lumen, and appears as a direct continuation of the pre-anal portion; its posterior tip is solid, and extends backward into the tail region for the distance of about one millimeter. From the ventral floor of this continuous intestinal tube, a median diverticulum leads backward and downward to the anus. The histological characters of this diverticulum differ markedly from those of the rest of the intestine, and by comparison with younger stages it becomes evident that the former has resulted from a proctodæal invagination. Where the intestinal tube is joined by the proctodæum the ventral portion, or cloaca, is T-shaped. The lateral arms receive the segmental ducts, and the ventral stem may be followed to the anus. In *Amblystoma*, then, the segmental ducts open into the intestine at the point where the proctodæal ectoderm and the entoderm pass over into each other. It is somewhat doubtful with which of the two germ layers the wall of the ducts becomes continuous; but it is possible that — in contrast to the condition obtaining in the *Anura* studied — the ducts open upon an ectodermal surface.

In the younger embryos of this stage, the walls of the pronephric tubules are all very thick; they gradually diminish in thickness as the embryo grows older. The lumen, on the other hand, is at first narrow, but afterwards becomes much wider. Its size varies greatly in different portions of the pronephros. For example, the lumen of the long arm of the common trunk, which forms the direct continuation of the segmental duct, is usually much narrower than the average lumen of the other pronephric tubules. The nephrostomal canals near their junction and the adjacent portion of the common trunk usually have a wide lumen. In the abnormal pronephros represented in Figure 57, however, the lumen of the first nephrostomal tubule was very narrow, a circumstance which, as I have already suggested, may possibly be correlated with the presence of a third nephrostome.

The lining epithelium of the tubes is composed of polygonal cells, which in the younger embryos have a high columnar form, but become gradually thinner as development proceeds. The nuclei when stained with Czokor's cochineal show a coarsely granular or reticulate structure, and are located close to the lumen of the tubule. The protoplasm takes a uniform delicate tint, which is masked, however, by the deeply staining yolk spherules. These are most abundant near the basal surface of the cell; they decrease in number and in size with the growth of the larva.

In the younger embryos of this stage, the somatopleure is composed of somewhat flattened cells, whose superficial dimension is approximately double the thickness of the cell. The walls of the pronephric tubules in these embryos have a thickness of about $37.5\ \mu$, while the parietal peritoneum has an average thickness of only about $15\ \mu$. These two epithelial layers are confluent at the nephrostomes, the wall of the tubule diminishing rapidly in thickness to that of the peritoneum. The nephrostomes, as well as many of the pronephric tubules, are slightly pigmented on their internal surfaces; but the pigmentation is by no means so conspicuous as in *Rana* and *Bufo*. In the older larvæ of this stage, the peritoneum is much thinner; but since the walls of the tubules have also diminished in thickness, nearly the same relations are to be observed at the nephrostome as in the younger embryos.

As in *Rana* and *Bufo*, the pronephric capsule in *Amblystoma* develops in the form of a downgrowth from the somatic layer of the protovertebræ. In *Amblystoma* the two-layered condition of the capsule and its connection with the overlying protovertebræ are maintained in the oldest larvæ of this stage. It seems probable, moreover, that the downgrowth from the protovertebræ is met by a more or less pronounced

upgrowth from the somatopleure immediately ventral to the pronephros. The thickness of the capsular sheath gradually diminishes in the course of the development of the larvæ, but it is in general approximately equal to that of the peritoneum in the same individual. In the older larvæ, moreover, the pronephros, and especially the segmental duct, become partially covered by a downward extension of the myotome. In such larvæ the anterior limb bud is prominently developed at this stage, and its cells cover in part the posterior ventral portion of the pronephros.

The sinuses within the capsule are bounded by mesenchymatic cells and contain scattered blood corpuscles; they are continuous posteriorly with the posterior cardinal veins, so that the venous blood in passing forward from the hinder portions of the body bathes the pronephric tubules on every side.

The vessel emerging from the anterior end of the pronephros receives a large vessel from the head, and from the point of union the ductus Cuvieri leads to the sinus venosus. The former vessel is one of the jugular veins. The distribution of this vein and its probable representative in the adult will be considered in connection with the following stage.

The first trace of the glomus appears in embryos of this stage. It consists, as in *Rana* and *Bufo* (compare Plate I. Figs. 8, 9, and Plate VI. Fig. 47), of a horizontal fold of splanchnopleure lying close to the dorsal angle of the body cavity. This fold extends, when fully formed, from the first nephrostome backwards to the second. The outer layer of the organ consists, as shown by its development, of splanchnic peritoneum, which is usually bounded within by a sharp contour. I am of opinion that the latter is in reality a thin structureless basement membrane. The interior mass of the glomus consists of several different elements. In the young stages embryonic blood cells form a prominent constituent. Other cells are present, which have an elongated form and are evidently connective-tissue elements; and there appear to be still other cells which are of a less modified character and in which nuclear mitoses occur. Many of the latter may well represent young stages in the development of blood corpuscles, for I have observed mitotic division of blood cells even in certain older larvæ of Stage VI. In addition to the classes of cells just mentioned, there are a few large cells whose nature is to me quite obscure. These cells measure $60\ \mu$ or more in diameter, and contain large yolk spherules, which are closely packed together and make up almost the entire substance of the cell. The histological characters of these cells ally them most closely with those of the entoderm, and in the youngest stages in which I have been able to identify

them they were closely associated with the yolk entoderm, which lies medio-ventral to the region of the glomus. It is probable that they arise from the entoderm and migrate into the interior of the splanchnopleural fold. I have been unable to find in either *Rana* or *Bufo* any cells similar to these large cells in the glomus of *Amblystoma*, and I have at present no suggestion to offer respecting their significance. The glomus, as I have already indicated, is a highly vascular organ, and even in the younger stages it is possible to find vessels which connect it with the aorta. These vessels usually follow the splanchnic layer quite closely, and appear to lie external to the large cells to which reference has been made.

In the younger larvæ of this stage the body cavity in the pronephric region has the form of separate chambers, from each of which a single nephrostomal tubule arises; but elsewhere the cavity is wanting on account of the contact of the peritoneal surfaces. In the older individuals it is expanded over a much larger area, but by the development of the lung bud a dorsal portion of the cavity is partially separated from the rest as a pronephric chamber.

STAGE VI.

Plate VII. Figs. 53, 54. Plate VIII. Figs. 61-65.

The larvæ included in this stage were taken from several different killings made in the course of three or four days. They measure about 9 mm. from the anterior end to the tip of the tail. An anterior limb bud is plainly visible upon surface view, and the tail is provided with a distinct membranous fin.

The great complication in the structure of the pronephros which is attained in this stage is accomplished by a continuation of the same process of forming convolutions that has been described for the preceding stage. Indeed, the separation of the two stages is at best quite arbitrary. Figures 61-65 represent various pronephridia of the present stage. It is to be noticed that the portion of the common trunk of which the segmental duct is the direct continuation can be traced from the anterior limit of the pronephros backwards without convolution, or after having formed a few insignificant loops. The common trunk from its junction with the nephrostomal tubules to this most anterior bend is thrown into a series of complicated convolutions, which may be so arranged as to present a gradation of considerable regularity (Fig. 62), or may be quite irregular (Fig. 65). In most cases, however, it is to be noticed that the arrangement of the loops is in general favorable for a compact

disposition of the tubes (Fig. 62). The convolution in this stage is no longer confined to the common trunk, the nephrostomal tubules undergoing slight contortion (Figs. 63-65).

I have determined the positions of the pronephric structures to the somites in these later stages by their relations to the spinal ganglia. The first and second nephrostomes lie very nearly in the same transverse plane as the first and second spinal ganglia respectively. In the youngest larvae of this stage the boundaries between the myotomes may still be made out in transverse sections, and the nephrostomes are then found to lie beneath myotomes III. and IV. It is probable that in later stages as well two myotomes occur in front of the first spinal ganglion.

The duct after leaving the pronephros pursues a nearly straight course backwards to the cloaca. In the larvae of this stage, the post-anal gut has atrophied, and the ducts open into the intestinal tract just at the point where it bends downward toward the anus or cloacal aperture. The outlets of the two sides of the body are quite widely separated, never opening into an unpaired median depression in the dorsal roof of the cloaca, as is the case in the corresponding stage of *Rana*. The outlets of the segmental ducts are situated between the eighteenth and the nineteenth spinal ganglion, which would correspond to somite XX. or XXI. Their position is, then, the same as in the preceding stage. (Compare page 254.)

In the series of embryos included under Stage V., it was shown that the walls of the pronephric tubules became gradually thinner as the animal developed. In the pronephridia of the present stage the same process has been continued, and the cells are frequently so reduced in thickness that the nucleus appears to be in contact with the basal as well as the superficial, or inner, surface of the cell. Occasionally tubes occur whose walls are so thin that each nucleus causes a protuberance into the lumen of the tube. But wherever the thickness of the epithelium exceeds the diameter of the nucleus, it is to be noticed that the latter lies close to the inner surface of the tube, whereas the yolk spherules are accumulated in the basal portions of the cells. The yolk spherules are much less numerous than in the preceding stage. In many cells they are wholly wanting, and in all they now form a much less prominent constituent than the cell protoplasm.

The nephrostomes present no new features of interest in this stage. Most of the pronephric tubules contain more or less pigment, which is usually accumulated in irregularly distributed dark patches. In one or two instances I have had a fair degree of success in dissecting out the

pronephros of a fresh specimen. In such an isolated pronephros the course of the tubes can be followed with tolerable accuracy in consequence of the pigmented areas occurring in their walls. The loss of yolk spherules, to which the pronephric tubes have been subjected on reaching the present stage, is shown in a striking manner by the transparency of the gland as contrasted with the snow-white yolk-entoderm.

The histological characters of the duct (Plate VII. Figs. 53, 54) resemble closely those of the pronephric tubules. Its calibre is greatest in the region immediately posterior to the pronephros (Plate VII. Fig. 54), becoming less as the duct passes posteriorly (Fig. 53). Throughout its course it is accompanied by the posterior cardinal vein (*vn. crd.*). In the older larvæ of this stage, the segmental duct in its passage backwards to the cloaca receives a large number of mesonephric tubules, which will be described in the sequel.

The pronephros of the present stage is covered on its dorsal surface by the main body of the myotomes. From the outer angle of each myotome, moreover, a distinct fibrillar sheet envelops the entire lateral surface of the gland. This layer is the capsule, whose origin has been discussed in connection with Stage V. In the present stage, it frequently becomes deeply pigmented.

The anterior portion of the pronephros is also overlaid by a stratum of smooth muscle fibres, which arises from the dorsal fascia. This muscular sheet is continuous in front with a muscle layer which is inserted upon the ventral surface of the mandible, and probably represents the depressor maxillæ of the adult.

The pronephros is also covered in part by the shoulder girdle, which in this stage is wholly composed of cartilage.

The vascular sinuses enclosed within the capsule are the direct continuations of the posterior cardinal vein. They also receive — usually about midway between the first and second nephrostomes — a blood-vessel, which may be traced nearly as far back as the cloaca, and which accompanies in its course the ramus lateralis of the vagus nerve (see Fig. 53, just median to *n. l.*). I am not aware of any prior mention of a vessel having this course, and am unable to state whether this vein has any representative in the adult.

The vessel emerging from the anterior end of the pronephros receives a vessel from the head, and the two form the ductus Cuvieri, which proceeds downward and inward to join the sinus venosus. The anterior branch may be traced forward into the head in the same direction as the original trunk; it accompanies in its course the ramus lateralis vagi.

In consequence of the uncertainty as to what vein of the adult this vessel represents, I shall here digress to describe its distribution at this stage. For purposes of description, I shall follow it from its point of junction with the cardinal vein forward towards its finer branches. Before reaching the ganglion nodosum, it sends a branch dorsalward, which can be traced for a short distance between the lateral wall of the cranium and the ganglion. The main trunk continues forward external to the ganglion, and gives off a branch which passes around the posterior end of the auditory capsule and enters the cranium. The original vessel now passes forward through a narrow channel left between the auditory capsule and the articulating portion of the mandibular cartilage. Near the anterior end of the auditory capsule it divides into two branches, one of which passes dorsal to the eyeball, accompanying in its course the ophthalmic branch of the trigeminal nerve; the other branch passes ventral to the eyeball, and continues into the anterior maxillary region, following the course of the canalis nasalis. The main trunk runs nearly parallel to the aortic root and its prolongation, the carotid artery, the efferent branchial trunks joining the aortic root by passing immediately ventral to the vessel whose course I have been following. The vein evidently corresponds to the one described under Stage V. of *Rana* (page 233, foot-note), and appears to me to represent in all probability the internal jugular of Gruby ('42) and of Ecker ('64-'82).

The glomus is considerably broader and thicker than in Stage V.; but its longitudinal extent is about the same. In the middle of its course its distal edge reaches across the body cavity and fuses with the somatic peritoneum which covers the pronephros. The structure of the organ appears to be nearly the same as in the preceding stage, but the interior mass is so compact that one can distinguish little more than the nuclei, which present quite uniform characters. Cells which are unquestionably endothelial are frequently evident along the basal surface of the peritoneal layer; they also traverse the interior of the glomus dividing this space into compartments. Pigment is present both in the peritoneal wall and in the interior mass. It has a scattered distribution, appearing in the form of perfectly black patches. The large cells to which allusion was made in Stage V. are present also in this stage. They have about the same size and histological features that formerly characterized them. The pronephric chamber has not changed materially from the condition exhibited in Stage V. The most anterior pronephric tubules are situated immediately lateral to a diverticulum of the body cavity, which in sections through this region appears wholly isolated. On following the

series of sections backward, however, the chamber enlarges greatly, even before the nephrostomes are reached, and is separated from the ventral portion of the body cavity only by the lung bud. Between the first and second nephrostomes, the pronephric chamber is divided into two parts by the fusion of the distal edge of the glomus with the somatic peritoneum covering the pronephros. Still farther posteriorly, an open communication is established, not merely between these two portions of the pronephric chamber, but also between the latter and the general body cavity.

In almost all the larvæ of this stage, the mesonephric tubules have appeared, and in many individuals they have already opened into the duct. There is always a space intervening between the pronephros and the mesonephros, in which no tubules are developed. This interval appears to be subject to some variation, but in the majority of cases it comprises four somites.

In the most anterior region of the mesonephros the tubules show traces of a metameric arrangement, but this is wholly lost in more posterior regions. These relations can perhaps be best illustrated by the accompanying table, which shows the positions of the right mesonephric tubules in the larva, whose pronephros is represented in Figure 64. The somites have been reckoned by reference to the spinal ganglia, but the results are here expressed in terms of the original metamerism of the myotomes.

| | | | |
|--------|---------|---------------------------|------------|
| Somite | III. — | Pronephric nephrostome I. | |
| " | IV. — | " | II. |
| " | V. — | Tubules absent. | |
| " | VI. — | " | " |
| " | VII. — | " | " |
| " | VIII. — | " | " |
| " | IX. — | 1 mesonephric tubule. | |
| " | X. — | 1 | " |
| " | XI. — | 1 | " |
| " | XII. — | 2 | " tubules. |
| " | XIII. — | 3 | " |
| " | XIV. — | 3 | " |
| " | XV. — | 4 | " |
| " | XVI. — | 5 | " |

Each tubule of the mesonephros (Plate VII. Fig. 53) has the ordinary form, which has induced several authors to call it "sickle-shaped," and consists of cells which are wholly devoid of yolk spherules, in which the nucleus occupies almost the entire body of the cell. Along the region which corresponds to the cutting edge of the sickle, a few loose cells (*fund.*

glm..) occur, which constitute the earliest fundament of the glomerulus. The nephrostomes, however, have not opened at this stage.

In the region between pronephros and mesonephros (Plate VII. Fig. 54) certain masses of cells are found on the median side of the duct in the same position as that occupied in the posterior region by the mesonephric tubules. These cells do not form a continuous mass, but are interrupted at intervals. The cords of cells thus formed do not, however, appear to correspond in their arrangement to the metamerism of the body. It is possible that they represent rudimentary nephridial tubules, but the evidence in favor of this interpretation must be regarded as far from satisfactory.

I have been unable to ascertain the precise mode of origin of the mesonephric tubules, having sought in vain for nuclear mitoses which should throw light upon this question. There are in younger stages many retroperitoneal (subperitoneal) cells which might be collected and rearranged so as to produce the tubules; or, again, the fundaments of the tubules might be formed by proliferation from the peritoneum. The cells of the tubule have evidently undergone very rapid division, as is indicated by the complete consumption of the yolk; and this circumstance seems to me to favor the second view. Furthermore, I have found nuclear mitoses (Fig. 54) in the region immediately in front of the mesonephros which indicate that the cords of cells in this region arise from the peritoneum. Although I am unable to assert that the mesonephric tubules arise from the peritoneum, I am inclined to regard it as probable that they do. There is no evidence, however, of a definite invagination of the wall of the body cavity.

This is the oldest stage of *Amblystoma* which I have examined, and with it I close the descriptive part of this paper.

III. General Discussion.

Having presented in a purely descriptive manner the facts of development as yielded by my own studies, I shall now endeavor to use these observations as a basis for the criticism of the results of other investigators, and in closing shall point out certain general conclusions which seem to me warranted by such a review.

Recent researches have extended greatly the number of animals in which a homologue of the pronephros is known, so that it may now be fairly assumed that the organ appears in the ontogeny of all Vertebrates.

In view of much recent evidence (Hatschek, '88^b, Rabl, '88, Ayers, '90)

which clearly supports the view that *Amphioxus* is closely related to Craniotes and occupies a position near the base of the Vertebrate phylum, the kidneys of this animal are of prime interest in the present connection. Notwithstanding the extreme importance of the subject, however, the relation of the excretory system of *Amphioxus* to other Chordates must still be regarded as a matter of considerable doubt.

At least seven different views have been advanced respecting the excretory organs of this animal. According to the earliest of these views, which originated with Joh. Müller ('42, p. 101, see also Langerhans, '76, p. 322, and Rolph, '76, p. 140), certain modified groups of cells lying in the posterior portion of the atrium are claimed to possess an excretory function. I presume that no morphologist would endeavor to homologize these excretory patches with the kidneys of Vertebrates. The same is true of the glandular structures described by Owen ('66, p. 533, Fig. 169, *h*), and the epithelial bands of Wilh. Müller ('75, p. 109). Nor can I see in the "pigmented canals," atrio-cœlomic funnels, of Lankester ('75, pp. 260, 261, and '89, pp. 394-397) any features which would definitely link them to Vertebrate nephridia.

The account given by Hatschek ('84) of his discovery of a single nephridium, which he believes to open into the pharyngeal cavity, is too brief to permit one to form a final judgment upon his interpretation. The observation has not been confirmed by any subsequent investigator save perhaps Lankester and Willey ('90, p. 459), who do not however regard this organ, which they call the sub-chordal tube, as a nephridium. There is nothing in its structure as described by either author which in my opinion justifies its comparison to a Vertebrate excretory tubule.

The most recent paper on this topic, which is by Weiss ('90), is of considerable interest from the physiological researches which it records: these show that a large portion of the atrial epithelium, as well as the excretory patches of Müller, have a well marked excretory function. Of greater morphological value is the description given by Weiss of certain small tubules in which the excretory function is peculiarly active. These tubules empty into the atrium at the upper margin of that cavity in the region of each secondary gill bar. They seem to project into the cœlom, but Weiss was unable to detect a continuity between their lumen and the cœlom. Since the relations of these tubules to the cœlom are not ascertained, I am of opinion that the observations of Weiss do not afford satisfactory reasons for regarding them as homologues of either the Vertebrate or the Annelidan nephridia. Weiss's account, however, is at least very suggestive. An important feature is the metamerism of the

tubules ; for while the metamerism of the gill bars does not correspond in the adult to that of the myotomes, yet we should not lose sight of the fact that according to Kowalewsky ('67, see his Figs. 36 and 39) such a correspondence exists in the embryo. At such a stage, then, there would be present a single excretory tubule for each myotome.

In a recent lecture before the Gesellschaft für Morphologie und Physiologie in München, Boveri ('90) has endeavored to show the existence in *Amphioxus* of homologues of the pronephros, the mesonephros, and the segmental duct. The tubules which Boveri regards as pronephric are probably the same structures as the excretory tubules of Weiss ; and I infer that the same have been seen by Spengel ('90, p. 282), though this writer makes no suggestion as to their significance. Both Weiss and Boveri claim to have proved by feeding the animals with carmine that the tubes are actually excretory. According to Boveri, also, they open into the atrium at the upper margin of each secondary gill bar ; but their course is somewhat differently described by the two authors. Boveri maintains that each tube communicates by means of several openings with the dorso-pharyngeal coelom. As confirmatory of his position that these canals represent the pronephric tubules of Craniota, he describes the relations they bear to the gill vessels, which he identifies with the segmental vessels described by Paul Mayer ('87, p. 343) in *Selachii*. According to Rückert ('88, pp. 239-242), the glomus of Elasmobranchs consists of a rete mirabile in connection with these segmental vessels. Adjacent to the excretory tubules, Boveri finds that the gills display an increase in vascularity, and that anastomoses are formed between the branchial vessels. This condition does not seem to have been noticed by Weiss. Spengel, who made a special study of the gill vessels, describes a longitudinal vessel at a corresponding level (longitudinal trunk of the ligamentum denticulatum), but does not discuss its significance. It seems to me that Boveri's observations, provided they be confirmed, afford fairly satisfactory evidence of the existence of true nephridia in *Amphioxus* ; and, as I shall endeavor to show in the sequel, that these are constructed on a type which may be assumed to represent a primitive condition of the Vertebrate kidney.

The starting point of Boveri's researches was the hypothesis that the atrial cavity and gonadal pouches of *Amphioxus* correspond to the segmental duct and mesonephros respectively of Craniota. The attempts of Haeckel ('74^a, p. 37, and '74^b, p. 305) and of Huxley ('76, pp. 221, 222) to discover a homologue of the segmental duct in *Amphioxus* must, in my opinion, be held to have at present merely an historical interest ;

it remains for me to consider whether the theory of Boveri be better grounded.

The arguments which are adduced in favor of the homology of the gonadial pouches and the mesonephros may be reduced to the following points of similarity. The gonadial pouches of *Amphioxus* are metameric diverticula of the dorso-pharyngeal cœlom, in accordance with the established views of Kowalewsky and Rolph, as confirmed by Boveri, who finds in the adult a continuity of the epithelia belonging to the two tracts; the mesonephric tubules likewise are primitively metameric diverticula from the dorsal portion of the body cavity (see Sedgwick, '80*, *et al.*). The generative cells develop in the walls of the gonadial diverticula; the early occurrence of germinal cells at the proximal ends of the forming mesonephric tubes has also been described by Rückert ('88, p. 257) for *Selachii*. Finally, the canal by which the gonadial pouches primitively communicated with the cœlom arches over the dorsal angle of the atrial cavity in a way that is very similar to that in which the mesonephric tubules curve outward to join the duct. The only reason — save those that require the prior assumption that the gonadia represent mesonephric tubules — which I can see for identifying the atrium with the segmental duct is the fact that nephridial (pronephric?) tubes open into it. This argument seems to me of very little weight. Boveri himself believes that the pronephros primitively opened directly to the exterior. Unless other evidence can be adduced, I see no adequate reason for regarding the formation of the atrial cavity as a step in the development of the segmental duct. On the other hand, that interpretation seems to me quite opposed to all that is known of the development of the segmental duct. As I have shown in the preceding pages, there can be no doubt that, in *Amphibia* at least, the duct develops solely from the mesoderm. According to the opposed view — the ectodermal origin of the duct — the development always proceeds from a *pair* of narrow rod-like thickenings of ectoderm, one on each side of the body, which are very different from the *unpaired* ventral groove from which, according to the most recent account (Lankester and Willey, '90) the atrium develops. If, now, we deny the homology of the atrium with the segmental duct, the outward arching of the gonadia becomes a most insignificant topographical resemblance. It seems to me that it would be manifestly unfair to base so far reaching a homology on the remaining points of resemblance, viz. the early occurrence of germinal cells in the mesonephric tubules, and the circumstance that the gonadia are metameric diverticula of the dorso-pharyngeal cœlom.

Turning now to Craniota, the pronephros in Amniota and Selachii is a wholly degenerate structure; in many Anamnia, however, it serves for a longer or shorter time as a functional excretory organ.

The pronephros of Dipnoi alone is wholly unknown. Beard ('90, p. 157) speaks of the transformation of a part of the pronephros into the Müllerian duct as "a well known fact"; but the only authority he cites in this connection (Parker, '89) does not make such a statement, nor have I succeeded in finding anywhere in the literature any account of the pronephros of Dipnoi. Unless Beard has personal observations on this matter, I believe that in Dipnoi absolutely nothing is known of the pronephros or its transformation, save such inferences as may be drawn from the adult anatomy. I shall therefore merely repeat the statement of Ayers ('85, p. 506), that the development probably proceeds as in Amphibia, since the adult urogenital system in this group presents the closest analogy with that of the Dipnoi.

The excretory system of Cyclostomes is similar to that of Amphibia. In Petromyzon a pronephros develops in the *Ammocætes* larva, but aborts in the adult. The number of nephrostomes and of tubules is small (4, according to Wilh. Müller; 4 to 5, Shipley; 3, Kupffer; according to Semon, an inner and an outer row of nephrostomes are to be distinguished); and they communicate with an anterior expanded portion of the body cavity. According to Fürbringer ('78^a, p. 42), the pronephros extends over about four somites. Opposite the nephrostomes, a vascular organ projects from the root of the mesentery into the body cavity. This is the so-called glomerulus; as figured by Scott ('81, Taf. IX. Fig. 24), it strikingly resembles the glomus of Amphibia. According to Scott, the pronephric tubules develop secondarily as outgrowths from the segmental duct. On the other hand, Shipley has confirmed the statements of Müller and Fürbringer, according to which the nephrostomes and tubules are formed by the incomplete closure of a longitudinal groove of somatopleure. Finally, Kupfer maintains that the tubules arise as *three separate evaginations of the somatopleure*, a result which is in harmony with my own observations on Amphibia.¹

In Myxine nothing is known of the early development; but in late stages an organ has been made known by the studies of Wilh. Müller

¹ In Goette's ('88, p. 163) preliminary account of the development of Petromyzon he states that a pronephros develops in the *same* manner as in Amphibia. This would indeed be a conclusion acceptable to me, but until the accounts are more at one in regard to the latter group the statement is somewhat vague. I await with interest the publication of that portion of Goette's final paper which relates to the excretory system.

('75) and of Fürbringer ('78^a, pp. 38, 39), which plainly represents the Amphibian pronephros. Whether it ever persists in the adult is still a matter of doubt (see Weldon, '84); but in young individuals, at least, the segmental duct (ureter) is prolonged anteriorly to the heart region. Here it gives off numerous coiled tubes, which branch and open by funnel-shaped nephrostomes into the pericardial cavity. On its dorsal side, the duct gives off a few tubules which terminate in glomeruli resembling those of the mesonephros. This condition and the large number of tubules constitute the main points of difference between the Amphibian pronephros and that of Myxine.

The pronephros of Teleosts and Ganoids appears to me to be reducible to a single type of structure, which can be easily derived from the condition present in Amphibia and Cyclostomes (and Dipnoi?). The so-called head-kidney of Teleosts described by Hyrtl ('51, p. 29) is probably derived from the embryonic pronephros, though mesonephric elements may also be found in the adult head-kidney (see Emery, '82, p. 46).

According to Rosenberg ('67, pp. 42 *et seq.*) and Oellacher ('73, pp. 97-100), the excretory organs arise as a pair of grooves of the somatopleure directly beneath the protovertebræ. A process of constriction, which proceeds from a middle region forwards and backwards, leads to the conversion of each groove into a tube, the segmental duct. The anterior portion becomes wholly cut off from the body cavity, and is thrown into numerous coils. The tip becomes considerably swollen, and is invaginated by an outgrowth from the aorta forming a single glomerulus on each side.

Goette's ('75, pp. 826, 827) account of the development of the pronephric glomerulus in Teleosts is somewhat different, and affords a better basis for homologizing the pronephros of Teleosts with that of Amphibia. Goette maintains that the somatopleural groove is imperfectly closed in front, leaving a single nephrostome, opposite which a glomerulus (glomus) is developed. Subsequently, the pronephric chamber becomes separated from the rest of the body cavity, and comes to resemble a Malpighian capsule with its contained glomerulus. While Fürbringer ('78^a) confirms Goette's view, Hoffmann ('86, p. 621 *et seq.*) has quite recently reasserted that this Malpighian capsule is the blind infolded end of the segmental duct, and the homology with the Amphibian glomus and pronephric chamber, which appears to me probable, he denies. Hoffmann's position does not seem to me tenable in the light of comparative studies. Even though it should be shown that the ducts

have absolutely no connection with the body cavity at the time when the glomerulus is formed, I could nevertheless defend my position by the assumption that the blind anterior end of the duct is a compound structure, representing both nephrostomal canal and pronephric chamber. It seems to me that, were it necessary to make this assumption, an extensive comparative study would justify such an interpretation.

The pronephros of Teleosts was long supposed to remain functional in the adult; but recent investigations seem to favor the conclusion that it never persists in fully mature individuals, with the possible exception of a few degenerate animals like *Fierasfer* (cf. Balfour, 81^b, '82; Groszlik, '85 and '86; Emery, '80, '81, and '85; Calderwood, '91).

The account given by Balfour and Parker ('82, pp. 415-424) of the development of the pronephros in *Lepidosteus* is in very close agreement with the development in Teleosts as described by Goette and by Fürbringer. The only conspicuous point of difference is, that, while in Teleosts the pronephric chamber becomes wholly detached from the body cavity, in *Lepidosteus* a remnant of the original communication probably persists as a so-called peritoneal tubule. As among Teleosts, the pronephros atrophies in adult *Lepidostei*.

Beard's ('89, pp. 114, 115) account of the early development differs greatly from that just given. According to this author, the pronephros is formed as a solid proliferation from the intermediate cell layer (Balfour) in the region from the 4th to the 8th or 9th somite inclusive. Externally, the proliferation fuses with the ectoderm. As a rule, there are formed three pairs of pronephric nephrostomes, of which the most posterior pair abort. The pronephric chamber is formed by the narrowing of the ciliated opening and the widening of the part opposite the glomerulus. Since Beard does not describe the development of the glomerulus, the account seems to me decidedly vague; but I believe I am right in accrediting to the author the view held by Hoffmann for Teleosts, that the glomerulus is not developed in the body cavity. As I understand him, it is developed in the course of the pronephric tubes.

All the studies on Ganoids thus far enumerated have been made upon *Lepidosteus*. In Acipenser. Salensky ('78, '80) maintains, in opposition to Kowalewsky, Owsjannikoff and Wagner ('70), that the excretory organs first appear as a differentiation in the form of a solid cord of cells. There is at that stage no trace of the coelom, nor of a division into protovertebral and lateral plate. Indeed, this cord of cells first marks the region where the latter separation will later occur. In its further development the

cord of cells acquires a lumen, either by a rearrangement of the cells, or by destruction of the axial ones. Anteriorly the structure now opens into the body cavity. The anterior portion elongates and becomes more and more convoluted up to the time of "post-embryonic" development. Opposite each of the peritoneal funnels are formed glomeruli [glomi] as processes from the radix mesenterii. They are covered by a pigmented layer of peritoneum. Salensky does not seem to me to have been very clear upon the earliest development, which was studied mainly by surface views, and I am of opinion that these stages would show very different conditions if more recent technical methods were employed. The most interesting feature of the development, as described by Salensky, is the occurrence of a glomus in the position which is typical for Amphibia and Petromyzon.

The excretory system has probably been studied more carefully in Selachii than in any other group. The independent researches of Balfour ('75 and '78) and Semper ('74 and '75) are in substantial accord, and have formed the basis for all subsequent investigations. For our purpose, the most prominent feature of the development as described by these authors is the absence of any structure which demonstrably represents the pronephros. According to Balfour, the first trace of the excretory system appears as a solid knob springing from the "intermediate cell mass" near the level of the hind end of the heart. From this anterior proliferation a solid cord of cells grows backward between ectoderm and mesoderm. The posterior portion is the fundament of the segmental duct; the anterior knob persists in adult females as the ostium abdominale of the oviduct. According to Balfour, this solid knob represents a rudimentary pronephros.

Very recently the early development of the excretory organs has been placed in a new light by the researches of Rückert ('88) and van Wijhe ('89). According to Rückert, the development begins with the formation of a pronephros as an outgrowth towards the ectoderm from the ventral portions of several *protovertebrae*, extending from the third or fourth trunk somite backwards for a distance of four to six somites. The thickening extends ventrally in each somite to the region where the segmented mesoderm passes into the unsegmented lateral plates. The proliferation, in the formation of which the somatic layer is alone concerned, shows on careful study a metameric character. From the posterior end of each *protovertebra* a narrow canal can be traced outwards and backwards, where it unites with a similar canal emerging from the next following somite. The pronephric mass fuses for a time with the

ectoderm and probably receives a contribution of cells from that layer. The duct grows backwards as far as the cloaca at the expense of the ectoderm. Having reached this stage of development, the pronephros rapidly degenerates. This process takes place in a slightly different way in the anterior and posterior regions. A variable number of the most anterior evaginations flatten out into a simple longitudinal groove of peritoneum, the ostium abdominale; the remaining ones become closed and detached from the peritoneum; thus there remains a longitudinal canal communicating with the body cavity by the slit-like ostium. In interpreting the structure as a rudimentary pronephros, it is important to note the discovery by Rückert (pp. 239-242) of a structure which he regards as a pronephric glomerulus, or glomus. This structure is developed in connection with segmental blood-vessels which pass from the aorta to the right subintestinal vein, and which have been described by Paul Mayer ('87, p. 343). In *Torpedo* the vessels are present on the right side in the same number as the segments of the pronephros, and as they pass ventrally between the ectoderm and the splanchnopleure it is to be noticed, in regard to the middle vessels at least, that they send out buds, which form projections from the median peritoneal wall opposite the pronephric tubules.

It will be at once seen that the development of the pronephros as described by Rückert is in striking agreement with the account I have given of the early stages in the development of the Amphibian pronephros, and I have no hesitation in homologizing the two organs. The earliest stage which has been observed in both groups is that which I have termed the pronephric thickening. This is followed in both by the stage of canalization; but the Selachian pronephros never goes beyond an early condition of the pronephric pouch, in which, however, the homologues of the nephrostomal tubules and the collecting trunk appear. The points of difference between the account I have given and that given by Rückert for corresponding stages of the Selachian pronephros seem to me, with a single exception, to be either unreal or insignificant. The exception to which I refer pertains to the participation of the ectoderm in the formation of the pronephric thickening. This condition I am confident does not occur in Amphibia. Moreover, the evidence upon which Rückert bases his statement seems to me far from conclusive, nor has his observation been confirmed by any subsequent investigator. Rückert described the pronephric thickening as a product of the proto-vertebræ. I cannot admit that this is true for Amphibia; but I believe that our differences of opinion are really due to the fact that we use dif-

ferent criteria for determining the boundaries of the protovertebræ. There can be no doubt that the earlier pronephric thickening is made up of metameric constituents; but I should be unwilling to regard all segmented mesoderm as belonging to the protovertebræ. On the contrary, I am of opinion that the ventral extent of the protovertebræ is for the first time defined when the longitudinal constriction appears which divides the primitive cœlom into protovertebral cavity and pleuro-peritoneal or (secondary) body cavity. When such a definite line of demarcation has been established, the remnant of the pronephros in *Selachii*, as well as the functional pronephros in *Amphibia*, remains connected with the latter space. The remaining points of difference relate to the number of tubules involved, — which, as we have seen, varies even within the class of *Amphibia*, — and to their position with reference to the somites. The latter feature seems to me to be at once difficult to determine and of minor importance.

Before the conclusion of this paper I shall endeavor to indicate how the glomus of *Amphibia* may possibly have been derived from the type of structure which is described by Rückert for *Selachians* and by Boveri ('90) for *Amphioxus*.

The results gained by van Wijhe ('89) do not seem to me to differ from those of Rückert in many respects which are of importance for a comparative study. The great divergence of their descriptions in the case of many details seems to me to be occasioned mainly by the peculiar conception which Rückert holds of the relations between the protovertebral and the lateral mesoderm. For these details and for the hotly contested questions of priority, I must refer to the original papers (van Wijhe, '86, '87, '88^a, '88^b, '89, Rückert, '88, '89), and consider here those features only which merit special attention because of their bearing on the general questions of homology. Van Wijhe denies positively the participation of the ectoderm in the formation of the pronephric thickening; and he claims that the ostium abdominale is formed from the pronephros by the fusion of the nephrostomes. Finally, structures which are supposed by him (pp. 480-482) to represent the pronephric glomeruli of Rückert are described as occurring on both sides of the body, not, as affirmed by Rückert, on the right side alone, and van Wijhe inclines to the view that they are actually equivalent to the glomi of *Amphibia*. The body described by van Wijhe consists of a vascular rod, which passes obliquely from the dorsal to the ventral lip of the pronephric pouch, and represents the last trace of the partition between two peritoneal openings, which have not yet fused. Rückert's description is not entirely

clear, and also suffers from misleading typographical and grammatical errors; but it is certain that the structure he describes lies within the splanchnic peritoneum, and is not to be confounded, as was done by van Wijhe, with the partition between two pronephric tubes. Rückert says ('88, p. 239), "Es [ein Paul Mayer'sches Quergefäß] zieht dicht an der medialen Grenze der Vornierenanlage vorbei und gelangt, indem es die Leibeshöhle durchbricht, d. h. ihre Wandung vor sich herstülpt, an die Aussenfläche des Darmes, wo es zwischen Ectoderm [soll wohl Entoderm heissen] und Splanchnopleura gelegen, mit der rechten Subintestinalvene confluiert." I cannot admit that the structure described by van Wijhe is the homologue of the Amphibian gloinus, nor do I believe that it corresponds to the structure observed by Rückert.

The mode of development of the excretory system is much alike in the three groups of Amniotes. It seems, however, best in the present instance to deal with the Reptiles separately from Birds and Mammals. The most important of the works on the Reptilian excretory system is perhaps the monograph of Braun ('77), which, however, is of little service in elucidating the earliest stages. Weldon ('83) first gave a satisfactory account of the early development. According to this author, the first trace of the excretory system in *Lacerta* is found in the region of the intermediate cell mass, and consists of a series of vesicles (Segmentalbläschen of Braun), which have a strictly metameric arrangement. Throughout a region of five protovertebrae (from the 8th to the 12th), there appears on the external wall of these segmental vesicles a rod of cells at first composed of discontinuous parts. This rod is the fundament of the segmental duct; in the region between two successive protovertebrae, it is budded off from the unmodified "middle plate" (Waldeyer), or intermediate cell mass. Behind the twelfth protovertebra, the duct grows backward, free from adjacent tissue. The rod of cells soon acquires a lumen, continuous anteriorly with the cavities of the segmental vesicles.

The observations of Mihalkovics ('85) upon *Lacerta agilis* differ from those of Weldon mainly in two particulars. In the first place, according to Mihalkovics (pp. 42, 43), the most anterior three or four pairs of segmental vesicles at the time of their origin communicate both with the body cavity and with the protovertebral cavity. In other words, they are formed as expansions of what I have termed the communicating canal, or *Mittelplattenspalten* of the German authors. Some somites in the series, however, may be without vesicles. Secondly, Mihalkovics (p. 48) maintains that the segmental duct buds off from the middle

plate as a *continuous* cord of cells at a time when only the first trace of the segmental vesicles has appeared. Before the (3 or 4) anterior segmental vesicles have entirely lost their connection with the body cavity, they communicate distally with the lumen of the segmental duct, and may therefore be regarded as typical nephrostomal canals. This condition is never encountered in the posterior vesicles, which develop independently of the coelom in the solid Wolffian blastema, or middle plate. In consequence of this difference in the mode of development of the anterior and posterior portions, Mihalkovics is of opinion that the first three or four segmental vesicles represent a rudimentary pronephros.

According to Strahl ('86), the segmental vesicles are budded off from the ventral portions of the protovertebræ, and gain secondarily a connection with the body cavity; the duct does not appear until the vesicles are evident.

Ostroumoff ('88^b, p. 81) confirms for *Phrynocephalus* the observations of Mihalkovics regarding the anterior segmental vesicles, although he is unable to ascertain the precise number that communicate with the body cavity. He also interprets these anterior vesicles as a pronephros. The duct, however, first appears in disjointed fragments lying between successive vesicles.

According to Hoffmann ('89), there develops in Reptiles a pronephros similar to that described by Rückert ('88) for *Selachii*. It appears as a series of evaginations of the somatopleure. These are formed in the region where the protovertebræ pass over into the lateral plates. The organ extends over a variable number of somites (6-7 in *Lacerta* and 5-6 in *Tropidonotus*). As protovertebræ separate from the lateral plate, the pronephric evaginations remain in connection with the former, except in the case of the first outgrowth (*L. agilis*, in *L. muralis* the first two), which forms for a time a single pronephric ostium. The most posterior outgrowth extends backwards, and forms the fundament of the segmental duct. The fate of the several evaginations is different. The most anterior and possibly the next following outgrowth abort at an early stage; the remaining evaginations become detached from the protovertebræ and fuse with one another, thus forming a tube closed in front, but continuous posteriorly with the segmental duct. Hoffmann identifies these evaginations with the segmental vesicles of Mihalkovics and Weldon, but asserts that these authors mistook for a separate fundament of the segmental duct a blind backward prolongation of the evagination belonging to the immediately preceding somite. These backward processes are described by Rückert for *Selachii*. Ostroumoff's ('88^b, pp. 78, 79) state-

ment, apparently unknown to Hoffmann, that the duct first appears in short fragments, each of which lies posterior to a segmental vesicle, could be readily brought into accord with these observations.

In regard to the correctness of Hoffmann's conclusions that these evaginations represent a pronephros, I am of opinion that there is considerable room for doubt. The organ described by Hoffmann differs in two important respects from that of Selachii, and from the young stages of the Amphibian pronephros as presented in the first part of this paper. In the latter groups, while the metameric evaginations are yet continuous with the coelom, they have also fused distally to form a longitudinal canal (collecting trunk); this condition I wholly miss in Hoffmann's account, according to which all the evaginations remain distinct from each other till they have entirely separated from the coelom, and only the more posterior outgrowths ever fuse together. Secondly, no structure comparable to the Amphibian glomus is described. The latter objection would apply equally to the account given by Mihalkovics.¹ None of the previous investigators were more successful in finding glomeruli of the pronephric type.

In regard to the former feature, however, the account of Mihalkovics is more satisfactory, since the most anterior three pairs of vesicles stand in precisely this relation to the body cavity and to the collecting trunk (segmental duct). In reviewing Mihalkovics's interpretation, Hoffmann says ('89, p. 272), since "die Vorniere als eine Ausstülpung, die Urnieren nicht als solche entsteht, kommt es mir höchst wahrscheinlich vor, dass die Vermuthung von Mihalkovics, nach welcher die proximalen Urnierenkanälchen der Eidechsen der Vorniere der Amphibien entsprechen, eine andere Deutung zulasse." I judge from this passage that Hoffmann is inclined to regard as mesonephric tubules the anterior three or four segmental vesicles described by Mihalkovics. I am quite unable to harmonize this view with Hoffmann's prior identification ('89, pp. 267, 268) of the pronephric evaginations described by him with the segmental vesicles of Mihalkovics and Weldon. The mode in which the mesonephric tubules develop in *Lacerta* is asserted to be very similar to that described by Rückert and van Wijhe for Selachii. If I properly understand Hoffmann's description, the space lettered *c.* in Tafel XVII. Figs. 3 and 4, is the lumen of a mesonephric tubule. From these figures it is evident that the mesonephric tubule develops from a portion of mesoderm ventral to the pronephros; but according to both Rückert and van

¹ Figures 18 and 19, referred to by Wiedersheim ('90b, p. 413) in this connection, do not relate to Reptiles at all. They represent sections of Duck embryos.

Wijhe, the mesoderm which produces the mesonephric tubules in *Selachii* belongs to a region dorsal to that which gave rise to the pronephros (see the diagrams appended to van Wijhe, '89, Taf. XXXII.).

In view of the difficulties to which I have alluded, it seems to me that Hoffmann's position cannot be regarded as satisfactory. Furthermore, if Hoffmann's observations¹ on the origin of the posterior mesonephric tubules be accurate, the contrast which Mihalkovics endeavored to establish between the anterior and posterior tubules does not exist. If, finally, these anterior three or four pairs of tubules develop in their course typical Malpighian capsules remote from the peritoneum, — Mihalkovics is not clear on this point, — I can see no reason for regarding them as pronephric. I am therefore of opinion that there is at present no evidence which *proves* a pronephros to exist either in *Lacertilia* or in *Ophidia*.

It remains for me to consider two recent papers by Wiedersheim ('90^a, '90^b), which describe a very interesting condition of the excretory system in *Crocodilia* and *Chelonia*. The anterior portion of the embryonic excretory organs in these groups consists of a number of tubules which take their origin in ciliated nephrostomes, and, after undergoing contortion, join a longitudinal canal continuous with the segmental duct. From the root of the mesentery a large glomus protrudes into the body cavity. It lies in a distinct fold of the peritoneum, and consists of a mass of highly vascular tissue receiving distinct vessels from the aorta. It extends continuously opposite a number of nephrostomes, and is evidently equivalent to the Amphibian glomus. In somewhat more posterior regions the conditions are essentially the same; but the nephrostomes and the glomus having approached each other, they are cut off from the main portion of the body cavity by a longitudinal fold of peritoneum. In this manner, there is formed a pronephric chamber comparable to that of *Amphibia*. In yet more posterior regions, the pronephric chamber with its contained glomus breaks up into a series of capsules containing glomeruli, each of which then appears to form the blind termination of a tubule. This is the region of the mesonephros with typical Malpighian capsules. In the subsequent development of the embryo, the anterior portion of this excretory system early atrophies, and the hinder part alone constitutes the well known Wolffian body, or mesonephros. In my opinion, the account given by Wiedersheim affords a satisfactory basis for the view that the most anterior portion of this excretory system is truly pronephric. It seems, however, quite impos-

¹ Similar observations are recorded by Orr ('87, pp. 325-327).

sible to draw a rigid line between pronephros and mesonephros. Indeed, such is a part of the conclusion which I think we shall finally be able to draw from the entire review.

The numerous accounts which have been recently given of the pronephros in the higher Amniota may be conveniently treated under three heads:—

(1.) According to Balfour and Sedgwick ('78, '79), the Müllerian duct in the Chick first appears in a region somewhat behind the front end of the Wolffian duct as three slender invaginations of the peritoneum which covers the Wolffian body. These invaginations later fuse at their distal extremities, and the most posterior involution grows backwards in connection with the Müllerian duct. There is thus formed a longitudinal canal with three peritoneal funnels, the whole structure being comparable to the pronephros of Amphibia. Slightly in front of the nephrostomes there is attached to the radix mesenterii a vascular body which resembles the Amphibian glomus. It receives blood-vessels from the aorta, and projects into the body cavity enclosed in a distinct sac of peritoneum. Gasser ('74, pp. 58, 59) had previously observed somewhat similar conditions in the anterior end of the Müllerian duct; and, by renewed investigation, Gasser and Siemerling were able to confirm the occasional occurrence of the phenomenon, though a single invagination appeared to be the rule. Multiple invaginations have also been mentioned by Kollmann ('82^b, p. 20), Siemerling ('82, p. 29), Janošik ('85, p. 43), and Mihalkovics ('85, p. 295); but Braun ('79) and Renson ('83, p. 37) were unable to find any evidence of such a condition. Braun also opposed Balfour and Sedgwick in their view respecting the nature of the vascular body, and Sedgwick ('80^b) later came to the conclusion that this structure was really a series of greatly modified mesonephric glomeruli. This interpretation was adopted by Balfour ('81^a, p. 590).

(2.) The second view is set forth in the recent account of Felix ('90), who describes in a chick embryo with eight protovertebræ a series of outgrowths, which, emerging from the lower hinder portions of protovertebræ IV.–VIII., extend backward and outward toward the ectoderm. The latter layer occasionally presents local thickenings in this region, and in some cases a connection between the mesodermal outgrowths and the ectodermal thickenings can be observed. In older embryos no trace of the structures can be found. As was the case with the evaginations found by Hoffmann ('89) in Reptiles, no fusion of their distal extremities is recorded. This condition makes them at once unlike the Selachian pronephros described by Rückert, and the early stages of the Amphibian

pronephros as detailed in the preceding pages. Moreover, Felix produces no evidence to show that they stand in any genetic relation whatever to the Wolffian duct, or to the pronephric structures described by other authors. In the present state of knowledge his interpretation seems to me untenable.

(3.) The remaining views all have the common feature that they regard certain rudimentary canals in connection with the anterior end of the Wolffian duct as pronephric. The views are somewhat divergent, but I have been able to compile from them a general statement which will in a measure explain their conflicts. In bringing the observations of each author under this general scheme, I shall frequently be driven to regard his results as incomplete, but I shall as far as possible avoid questioning his statements from an *a priori* standpoint.

In general three regions of the embryonic excretory organ may be distinguished: the pronephros, an intermediate region, and the mesonephros. For criteria of these regions, I shall use in the main glomerular structures: those of the pronephros are glomi wholly external to the tubules; those of the intermediate region are transitional glomeruli, which develop in peritoneal canals, but project through the nephrostomes into the body cavity; those of the mesonephros are typical glomeruli, which have only a mediate connection with the body cavity through the tubule.

It now remains to consider the results of the observers whom I have placed in my third group. The work of Gasser and Siemerling ('78, '79), subsequently carried on by Siemerling ('82), relates to Birds alone. These authors recognize two distinct portions of the Wolffian duct: a portion lying in front of the fifth somite, and a posterior portion. The former shows many irregularities, is broken up into discontinuous fragments, and early atrophies; the latter develops more slowly, but more regularly, and persists as the duct of the Wolffian body. The first indications of tubules consist of the so-called primary cords, which are continuous with the coelomic epithelium by means of funnel-shaped ostia, while they are distally in contact with the duct. Gasser and Siemerling maintain that they belong to the most anterior part of the mesonephros, a portion which early atrophies. They are quite similar to the S-shaped canals of Kölliker ('79). In front of the region of the "primary cords" similar evaginations occur, but these never reach the duct. A typical glomus, which may be single or may be divided into parts, projects from the radix mesenterii opposite the openings of these evaginations. In embryos of this stage the space between the most anterior Wolffian tubule and

the pronephric structures is traversed by a series of glomeruli which resemble most closely those of the mesonephros. Siemerling calls them transitional glomeruli. The pronephros of our scheme would be represented in this account by the region in front of the fifth protovertebra; the intermediate region would correspond to the space occupied by the transitional glomeruli, and also, as I believe, to that previously occupied by the primary cords; the mesonephros would form the rest of the organ.

According to Sedgwick's ('81) account of the development in the chick, the Wolffian duct, in separating from the proliferation in which it arises (region between the 7th and 11th protovertebrae), remains connected with the peritoneal epithelium by short cords of cells. Between the 8th and 15th protovertebrae, the duct, as it grows freely backwards, comes secondarily into contact with such a cord of cells in each somite. Behind the 15th somite, the fundaments of the tubules (intermediate cell mass) do not join the duct until their differentiation is somewhat advanced. The cords of cells in the region between the 7th and 11th protovertebrae acquire lumens which may be continued even into the duct; but both cords and duct soon entirely disappear. Although no glomus is described, this region probably represents the pronephros. Between the 12th and 15th protovertebrae typical nephrostomal funnels are formed, in which transitional glomeruli develop. This portion of the organ would then correspond to the intermediate region of the general scheme; behind this region comes the typical mesonephros. Sedgwick regarded the first mentioned region as pronephric; but he hoped to be able to harmonize such a view with the position (*cf.* page 276) formerly taken by himself and Balfour (Balfour and Sedgwick, '79).¹

In the foregoing description I have assumed that the most anterior portion of the Wolffian duct and the accompanying transverse canals observed by Sedgwick corresponded to the pronephric region as described by Siemerling. This interpretation seems to me in all probability correct; yet it should be recalled that the pronephros described by Siemerling lies in front of the 5th somite, and is anterior to the region in which the early proliferation to form the duct took place; whereas,

¹ Mihalkovics's statement, that Sedgwick abandoned his former view, is incorrect, as will be seen by referring to the closing paragraph of his article (Sedgwick, '81, p. 468).

In the second edition of Foster and Balfour's ('83, p. 218) *Elements of Embryology*, revised by Sedgwick and Heape, the anterior end of the Müllerian duct is the only homologue of the Amphibian pronephros suggested.

the pronephros, according to Sedgwick, lies between the 7th and 11th protovertebræ and arises in the same region in which the duct first appears.

Lockwood ('87, pp. 657-663) describes three regions in the embryonic excretory organ of the Rabbit. In the most anterior region (pronephros), the duct consists of isolated fragments, which are connected with the body cavity by 2-3 nephrostomes. Then follows a region of typical nephrostomal canals with glomeruli, and finally typical blind mesonephric tubules. Possibly the last two regions belong to the mesonephros; but in none of the accounts of Mammalian development have I been able to recognize with certainty the intermediate region.

According to Renson ('83, p. 29), glomeruli develop in the Chick in the region of the pronephros, which is otherwise described in agreement with Sedgwick's account. The pronephric tubules atrophy with the exception of their nephrostomes, and in the hollow of each funnel there appears a glomerulus which soon comes to project freely into the body cavity. In a region directly posterior to that in which the free glomeruli occur, there are found the so-called mixed glomeruli, which are situated in the base of an infundibular depression, and are partially covered by a fold of peritoneum. This, as well as the more anterior portion of the system, Renson regards as belonging to the pronephros. He also describes in the Rabbit a series of peritoneal involutions in connection with a discontinuous duct. In this region he likewise observed a vascular structure, which he regarded as a very rudimentary external glomerulus. A similar observation has been recorded for human embryos by Lockwood ('87, pp. 662, 663), and for *Arvicola* by Spoor ('83, p. 86, footnote). It is difficult to arrive at a satisfactory estimate of Renson's position. There would be no difficulty in classing him with Sedgwick, were it not for the circumstance that he describes for the pronephric region (6 or 7th to 11 or 12th somites) glomerular structures which, according to his own comparison, develop in the same way as the transitional glomeruli observed by Sedgwick in the "intermediate" region only (11th to 14th somite). If, however, it should prove to be true that only the "mixed" glomeruli develop in this way, the conflict would at once be removed, and Renson's account would show the three primary regions in their typical condition.

According to Mihalkovics the most anterior two or three tubules (4-7 somites) in the Chick and Duck are derivatives of the communicating canals, and gain a connection with the duct while yet opening into the body cavity by a distinct ostium. The posterior canals, on the contrary,

are all differentiated from the solid "Wolffian blastema," and never have any connection with the body cavity. Posterior to the last pronephric canal, 5-6 free glomeruli are to be found. The anterior canals form much earlier than the posterior, indeed they wholly abort before the mesonephros attains its final development; and they together with the free glomeruli are, in his opinion, to be regarded as equivalent to the pronephros and glomus of Amphibia. Mihalkovics also mentions the occurrence of transitional glomeruli; these are typical glomeruli which lie near the peritoneal covering of the Wolffian body. It seems to me probable that these glomeruli really belong to the mesonephros, and that at least a portion of the "external glomeruli" belong in reality to the class which I have designated transitional glomeruli. This interpretation would not merely be in agreement with the described position of the glomeruli with reference to the somites, but it would also accord well with the figures Mihalkovics gives of the two sets of glomeruli. Thus, in his representation of a transitional glomerulus (Taf. I, Fig. 17, *g. m.*), there is little reason to regard the structure as in any way different from a mesonephric Malpighian body. I may here further remark, that nearly all other modern investigators agree in deriving a part, if not all, of the mesonephros from a layer of cells which primitively bounded the cœlom, rather than from a strictly indifferent blastema. In this light, the validity of the principal contrast Mihalkovics sought to establish between the pronephros and mesonephros becomes at least very uncertain.

The account of Janošik ('85) affords the best basis for the general scheme I have proposed. The most anterior region, or pronephros, develops somewhat *later* [!] than the first tubules of the mesonephros (primary cords?). The duct in the region of the pronephros is broken up into fragments, which receive rudimentary peritoneal canals. Three typical glomi are developed on the radix mesenterii. In the next following region (intermediate), from two to five peritoneal canals communicate with the Wolffian duct. Near the nephrostomal ends of these canals transitional glomeruli develop. Both the pronephros and the intermediate region rapidly atrophy. The remaining portion of the embryonic excretory organ is the true mesonephros. The mesonephric tubules are either developed as separate buds from the peritoneum, or are differentiated from a blastema which is directly derived from the peritoneum. Janošik was able to confirm Renson's discovery of rudimentary pronephric tubules in the Rabbit, but was unable to find in this form any trace of external glomeruli. Later, however, he ('87, p. 582) described in a young human embryo, 3 mm. in length, a peculiar projection into the

body cavity. The structure resembled the external glomerulus of Birds, and he was inclined to interpret it as such in this case.

In the preceding pages I have endeavored to present a comprehensive résumé of the development of the pronephros as described in groups of Vertebrates other than Amphibia. In this review it has been shown that an equivalent of the Amphibian pronephros has been claimed to exist in all Craniota; and that a mode of development similar to that described in the early part of the present paper has been found in Selachii by Rückert ('88) and van Wijhe ('89), in *Petromyzon* by Kupffer ('88), and in *Lepidosteus* by Beard ('89). The Reptilian pronephros as described by Hoffmann ('89), and that of the Chick according to the account of Felix ('90), do not seem to me to be in perfect accord with this mode of development.

It now remains for me to compare the results of my studies, as detailed in the descriptive part of the present paper, with those which have been recorded by other writers on the development of the Amphibian pronephros. According to the account which at present receives most general acceptance, the pronephros first appears as an outfolding of the somatopleure in the form of a longitudinal groove. The anterior end of this groove is destined to become the pronephros; the remaining portion is constricted off to form the segmental duct. Since the process of constriction advances from before backwards, stages may be found in which a completed tube is continuous posteriorly with a mere groove of the somatopleure. In the anterior region, the groove remains in communication with the body cavity, and grows down towards the ventral surface of the embryo in the form of a broad pocket. The slit-like peritoneal opening of this pouch closes throughout the greater part of its length, leaving, however, two or three regions of incomplete closure, the fundaments of the nephrostomes. The nephrostomal tubules are formed by the fusion of the walls of the pouch between two nephrostomes. The regions of fusion extend in vertical lines from the nephrostomal margin of the pouch nearly to its ventral border, where there is left an unfused and therefore continuous longitudinal tract constituting the canal which I have called the collecting trunk. This view of the development of the pronephros, although suggested by Wilh. Müller ('75), was first described in detail by Goette ('75) for *Bombinator*, and was later extended to other Amphibia by the researches of Furbringer ('77). It has been entirely confirmed by Wichmann ('84), by Hoffmann ('86), and still more recently by Marshall and Bles ('90a).

In opposition to this view, I would maintain: (1) that the first trace of the excretory system consists of a solid proliferation of somatopleure, the pronephric thickening; (2) that the lumen of the system arises secondarily; and (3) that the pronephric tubules do not appear in consequence of the local fusion of the walls of a widely open pouch, but that they are differentiated at an early stage from the hitherto indifferent pronephric thickening.¹

The development of the pronephros and duct from a solid mass of mesoderm was a common feature in the accounts of those who wrote prior to Wilh. Müller and Goette, but since then this mode of origin, though repeatedly maintained by single observers, has failed to gain general acceptance. Clarke ('81) described a solid pronephric thickening, and asserted that the lumen arose secondarily in this mass; the details of the process are, however, not accurately given. Duval ('82) also described the pronephros as first appearing in the form of a solid thickening. He however states that it later acquires a slit-like opening into the body cavity, and that by the imperfect closure of this opening the successive nephrostomes are formed, as described by Goette and Fürbringer. This latter statement I am unable to confirm. Gasser's ('82, pp. 89-97) short note gives, on the other hand, an account of the early development in *Alytes*, which is in substantial agreement with my own observations. His account of the first differentiation of the nephrostomal canals is not very full, but it is not improbable that he conceived it to take place in a manner altogether similar to that which I have described. His statements seem to me in general correct,² but incomplete.

Janošik ('85, p. 19) states, on the basis of personal observations, that the first trace of the segmental duct in *Bufo* and *Triton* is a solid mass of cells, which he is, however, inclined to regard as a disguised fold of somatopleure.

According to a recent account by Kellogg ('90), a lumen does not appear anywhere in the organ (except in the region of the nephrostomes) until it has been separated from the peritoneum. Finally, Mollier ('90, Rückert and Mollier, '89) has published an account of the early development, which is for the most part in close accord with the results of my own studies. Since these results were gained entirely independently of

¹ The large cavity which the pronephric pouch presents in Stage IV. of *Rana* and *Bufo* is a secondary condition produced by the expansion of the lumens of the several diverticula.

² I must here except his statement that the second tubule is differentiated before the rest; this I believe to be an error.

Mollier's researches and were written out before his paper came into my hands, it seems to me that my confirmation of his position affords excellent evidence of the correctness of the view advocated. In one feature alone our accounts of the earliest condition of the pronephros would seem to differ widely, but I am confident that the difference is apparent rather than real. Mollier states that each of the diverticula which form the first indications of the nephrostomal canals emerges from a protovertebral cavity. This statement, as I have already shown, does not in my opinion accurately represent the actual conditions. In the stage under consideration, the dorsal portion of the mesoderm is in the anterior region divided by transverse planes into a series of metameric blocks; the pronephric thickening also is made up of metameric constituents, and is continuous dorsally in *Amblystoma* with two, in *Rana* and *Bufo* with three, of the blocks of mesoderm. As yet no definite line can be drawn between the protovertebræ and the lateral plates; in a slightly older embryo, however, the protovertebræ begin to be constricted off from the lateral plates, and it is at once evident that the pronephric tubules have to do with the ventral segment of the mesoderm. This difference in our accounts seems to me then very trivial, and my only excuse for dwelling upon it is the circumstance that Rückert and Mollier seem to attach great morphological significance to this feature of their account. This relation to the protovertebræ seems to me quite untenable.

Previous authors have been singularly reticent respecting the exact position of the pronephros with reference to the body somites. Fürbringer ('78^a, p. 5) states that the pronephros of *Anura* extends over three, that of *Urodela* over two somites; but I have looked in vain for a statement which should show whether the nephrostomes are segmental or intersegmental in position. Kellogg states that each nephrostome occurs opposite the middle of a protovertebræ. Marshall and Bles confirm this statement, and contend that, in the case of *Rana*, the nephrostomes lie in the 2d, 3d, and 4th somites behind the auditory vesicle. According to Mollier ('90, p. 213) the pronephros appears in *Triton* in the region of the 1st and 2d trunk protovertebræ; but since the most anterior two protovertebræ are reckoned to the posterior region of the head, these represent the 3d and 4th protovertebræ of the series. The enumeration which I have given for *Rana* and *Bufo* is in precise agreement with that of Marshall and Bles. For *Amblystoma* my account is in agreement with that of Mollier for *Triton*.

I am not aware that any definite attempt has thus far been made to ascertain which of the three nephrostomes of *Anura* is unrepresented in

Urodela. At first sight it would seem probable, — and by implication I accredit this opinion to Mollier, — that the rudimentary third tubule occasionally present in Urodeles corresponds to the normal third tubule of Anura. This view, however, is not in precise harmony with the relations of the nephrostomes to the myotomes. As I have already shown, the first nephrostome in *Amblystoma* is situated beneath myotome III., whereas in *Rana* and *Bufo* it occurs under myotome II. If now the enumeration of the somites in the two cases correspond, it follows that the first and second nephrostomal tubules of *Amblystoma* are equivalent to the second and third tubules respectively of *Rana* and *Bufo*, not to their first and second tubules, and that the occasional rudimentary third tubule of Urodeles belongs to a more posterior somite, and is unrepresented in Anura. In *Amblystoma* the root of the vagus nerve arises immediately in front of the somite which I have denominated I.;¹ the same is true in the case of *Rana* and *Bufo*, and I am inclined to regard these as equivalent somites. It is possible that somite II. of *Amblystoma* is not represented in *Rana* and *Bufo*; but this is hardly probable, since it belongs to the head region, which is hardly likely to vary in such closely related groups, and since it is evident that the greater number of protovertebrae present in Urodeles as compared with Anura is largely accounted for by additional protovertebrae in posterior regions, particularly in the region of the mesonephros, as I believe. In general, it seems to me that we should be more ready to admit the abortion of the most anterior tubule in Urodela than to assert the existence of an additional protovertebra in Anura.

All the more recent writers are agreed that in Anura three pairs of pronephric nephrostomes occur, Giles ('88, p. 135) alone claiming that a degenerating pronephros may have four. In Urodela the typical number is two; but Mollier ('90, p. 224) has recorded the occasional occurrence of three pairs in Triton, and I have made similar observations in *Amblystoma*. Spengel ('76, p. 19, Taf. II. Fig. 21) maintained, on the evidence of a specimen in which the pronephros was largely degenerated, that four pairs occur in Cæcilia; the recent observations of Semon ('90, p. 462), on the other hand, have shown that there exist in Ichthyophis on *each* side of the body ten *pairs* of nephrostomes, therefore forty in all.

¹ Somite I. of this enumeration probably corresponds to the one which has been called somite XI by Houssay ('91). Houssay believes that he can identify in Amphibia the somites which have been observed in the head region of Selachii. If his conclusions are accurate, they are evidence in favor of the view that this region of the body is very permanent.

Of the two nephrostomes belonging to any pair, one opens freely into the body cavity, the other communicates with a pronephric chamber, which contains the glomus and is completely shut off from the body cavity. The meaning of this condition I shall consider in the subsequent discussion. The pairs of nephrostomes on each side are slightly more numerous than the overlying protovertebræ.

The origin of the so-called ventral part (common trunk) of the pronephros has recently become the subject of controversy. According to Goette the duct at first communicates with the posterior end of the widely open pronephric pouch. At the same time that the nephrostomal canals are formed by local fusions of the walls of the pouch, a similar process constricts off the posterior ventral portion of the pouch; this has the effect of lengthening the duct, so that the point of its attachment is carried forward to the place where the converging nephrostomal tubes unite. The portion of the longitudinal canal in front of the most posterior nephrostome represents the "ventral part" of the pronephros.

According to Fürbringer, the longitudinal groove which forms the earliest fundament of the pronephros and duct becomes entirely constricted off from the somatopleure as far forward as the opening which leads into the pronephric pouch; this slit-like opening then elongates posteriorly, so as to extend into the region formerly occupied by the longitudinal canal alone; the latter thus comes to lie ventral to the last nephrostomal canal, and forms the ventral part of the pronephros.

Kellogg ('90) opposes the accounts of previous observers, and claims that the ventral part "is formed from the ventral side of the dorsal part of the pronephros, and *anterior to the last nephrostome*." Marshall and Bles, alluding to Kellogg's description, declare that it is in exact accordance with the accounts of Goette and Fürbringer. I have not been able to satisfy myself as to the precise manner by which Kellogg conceives the formation of the ventral part to have taken place; but I think he has said enough to contrast his position strongly with that of Fürbringer, according to whom the ventral part of the pronephros first appears as a portion of the somatopleural fold immediately *posterior* to the part which gives rise to the nephrostomal canals. Kellogg argues, however, that, were the views of previous authors correct, some portion of the pronephros would appear behind the last nephrostome; but this is actually never the case. The force of this argument I am wholly unable to appreciate, and I must in consequence feel some doubt as to whether I have properly interpreted Kellogg's previous statements.

According to Mollier, the "ventral part" is differentiated in the

ventral portion of the broad pronephric thickening. Mollier's description is substantially in accord with my own observations, and it seems to me probable that Kellogg's statements are to be understood in the same way.

The structure of the functional pronephros was early the occasion of much controversy. The discoverer of the organ, Joh. Müller ('29 and '30), describes and figures it as a cluster of blind tubules, which radiate in the form of a rosette from the anterior tip of the segmental duct. This view was shared by the larger number of the early investigators. According to von Wittich ('52), the gland is typically formed by the convolutions of a single tube; in the more complicated pronephridia, however, this canal may give off branches. It is to Goette and Fürbringer that we owe the first accurate account of the process of convolution.

According to these authors, the gland is composed of two portions: a "dorsal part" (collecting trunk and nephrostomal canals), which alone receives the nephrostomes,¹ and a "ventral part" (common trunk), which serves as the efferent canal, and is in communication with the anterior end of the segmental duct. Both ventral and dorsal parts undergo extensive convolutions, and give rise to blind diverticula. Subsequent authors have in general confirmed Fürbringer's account, but have added no new matter to the description. Selenka ('82) describes and figures an interesting condition of the pronephros in *Hylodes*. The glands of the two sides are unsymmetrical, and depart widely from the typical structure known in *Amphibia*. Following the nomenclature which I have proposed in the descriptive part of this paper, it is evident that the nephrostomal canals and the collecting trunk are present, but do not show the convolutions customary in these parts. The "ventral part" of the gland, however, is not formed by the windings of the common trunk, but is composed of great irregular blind pouches which communicate with the collecting trunk, while the latter opens *directly* into the anterior end of the segmental duct. This condition of the pronephros evidently represents the degeneration of the gland, and Selenka is inclined to correlate the premature appearance of this complication in *Hylodes* with the absence of gills in the larvæ of this form.

Kellogg has studied the structure of the pronephros in *Amblystoma* and *Rana* by means of reconstruction from cross sections. His pre-

¹ Duval ('82, Fig. 7), figures the second pronephric nephrostome in *Rana* as opening directly into the ventral part of the gland. I have never seen such a condition in my preparations, nor do I know of similar observations being elsewhere recorded. It seems likely that Duval has here fallen into error.

liminary notice, however, does not describe the process of convolution in detail. An interesting feature is the statement that blind diverticula do not appear until the tubes of the gland have become very much convoluted. In the pronephridia which I have studied, I have never seen a blind diverticulum. My observations do not extend to sufficiently old stages to allow me to deny that such diverticula appear anywhere in the developmental history of the gland, but the organ can reach at least the high degree of complexity shown in Figures 41 and 65, and yet be composed of the windings of the nephrostomal canals, the collecting trunk, and the common trunk without possessing any blind diverticula.

It is needless for me to discuss in this place the histology of the tubular portion of the pronephros. These details have little general interest, and they have furthermore been accurately given by Fürbringer and Hoffmann ('86).

The dilated chamber which I have described (page 240) was also observed by Hoffmann, but he was unable to determine what portion of the system was concerned in its formation. Similar dilated chambers are likewise described by Marshall and Bles, who regard them as steps in the degeneration of the tubules. The early appearance of these dilated regions in *Rana* (see page 232) seems to me to render this interpretation improbable.

According to the usual account, the capsule arises as a differentiation of the connective-tissue stroma, which lies between the pronephros and the ectoderm. Duval ('82, pp. 25, 27) alone has claimed an origin from the overlying protovertebræ; but, singularly, his statement has been wholly neglected by subsequent writers. His observations on this point agree in all essential features with my own.

The glomus was discovered by Joh. Müller ('30, p. 12), but the significance of the structure was wholly problematical until Bidder ('46, p. 58) suggested its glomerular nature, which has since received general acceptance. This view has, however, been opposed by Semper ('75, pp. 439 *et seq.*), and more recently by Hoffmann ('86, pp. 572, 573). According to Goette and Fürbringer, the glomus arises as an outfolding of the splanchnopleure opposite the pronephric nephrostomes. The interior of the fold becomes occupied by mesenchymatic cells and with blood tracts, which communicate with the aorta. According to Hoffmann, the interior is largely occupied by "columns" of large cells, which would seem foreign to the nature of a glomerular structure. These "columns of cells," he says, may be seen to arise, in *Bufo* at least, by the invagination of the superficial covering of the glomus. I have myself seen continuous cylin-

dricul cords of cells in the glomus ; but in most cases I have been readily able to satisfy myself that this appearance had to do with densely packed blood cells lying in a definite vascular tract. I have also occasionally met with invaginations of the superficial (peritoneal) epithelium of the glomus (page 247) ; but it seems to me, even should it be shown that they give rise in the interior to columns of cells, that this would not be a very serious objection to the view which ascribes to the organ a glomerular function. In favor of that view, many arguments may be adduced : (1) the highly vascular nature of the glomus ; (2) its position in an open chamber of the body cavity directly opposite the pronephric nephrostomes ; (3) its serial relations with the mesonephric glomeruli ; (4) its appearance and degeneration synchronously with the pronephros ; and (5) the circumstance that its homologue, wherever found in other classes of Vertebrates, is always in equally close relation with excretory tubules. The last argument seems to me the most weighty, and I am of opinion that a comprehensive comparative study proves beyond question the glomerular nature of the structure.

In the descriptive part of this paper I have stated that, in satisfactory sections through the blood-vessel which leads from the aorta to the glomus, one could frequently observe that the ramifications within the glomus did not appear to be terminal, but that the vessel seemed to give off a lateral branch to the glomus, while the main trunk continued on toward the ventral side of the body. An explanation of this condition has occurred to me, which, if confirmed, will be of considerable morphological significance, though at present I can merely offer it as a suggestion. As we have already seen, the glomus of *Selachii*, according to Rückert ('88, pp. 239-242), does not receive a separate blood-vessel directly from the aorta, but a rete mirabile is developed in connection with the segmental vessels described by Paul Mayer. I have not succeeded in tracing the main aortic branch to the ventral side of the larva ; but, as far as it could be followed, the course of the vessel between splanchnopleure and entoderm corresponds perfectly with that of one of the segmental vessels described by him. It seems to me quite possible that, in *Amphibia*, the dorsal portion, which is in communication with the glomus, is the only part of these rudimentary vessels which is retained, and that the remaining portion, having ceased to be of functional importance, fails to develop.

Having completed my survey of our knowledge of the development of the pronephros in the several classes of Vertebrates, I now turn to a

consideration of the development of the segmental duct. As is well known, observers up to a very recent date have been almost unanimous in ascribing a mesodermal origin to this structure. In regard to the details of the process, however, they have been less at one; and I shall accordingly treat of their accounts under three heads, which seem to me to represent fairly well marked phases of opinion.

According to one view, *the duct arises as an evagination of somatopleure, its lumen being therefore a detached portion of the body cavity*. Such a mode of origin was advocated by Rosenberg ('67, pp. 42 *et seq.*) for Teleosts; and this feature of his account has gained almost universal acceptance both for Teleosts and for Amphibia, having been recently entirely confirmed by Hoffmann ('86) and Hemmery ('88, '89). According to Wilh. Müller ('75) and Fürbringer, the duct arises in this way also in Petromyzon, and a similar claim has been made for Ganoids by Kowalewsky, Owsjannikow, and Wagner ('70), and by Balfour and Parker ('82). In Selachians, however, the weight of the evidence is distinctly opposed to this view, and I am not aware of its having been advocated by any one besides Schultz ('75).

In Amniotes also such an account of the early development has not received general acceptance; it was first claimed in this class by Romiti ('74), and was adopted, with some modification it is true, by R. Kowalewsky ('75), and by Dansky und Kostenitsch ('80, p. 24). Very recently such a mode of origin has been reasserted by Fleischmann ('87) for Carnivores and the Duck.

My own observations on Amphibia indicate that in this group the duct does not arise as a fold; and I am of opinion that, in both Cyclostomes and Ganoids, the evidence that the duct arises by evagination is at present unsatisfactory. It seems to me probable, on the contrary, that the method of origin which is usually recognized as characteristic of all the Anamnia with the exception of Selachii exists, if at all, only in Teleosts. In view of the peculiar obstacles which Teleostean material presents for embryological study, one should be cautious in affirming for this group a mode of development which, in my opinion, is not proved to exist in any other class of Vertebrates.

A second view of the origin of the duct is, that it *arises from a solid proliferation of somatopleure*. According to Fürbringer ('78*), Spoor ('83, p. 84), and the earlier writers (Remak, '55, Kölliker, '61, Bornhaupt, '67, Waldeyer, '70, and Foster and Balfour, '74), the duct arises in the chick by a proliferation *in situ* of the subjacent mesoderm, and a similar origin is maintained for Petromyzon by Scott ('82). The more recent

view, however, affirms that the posterior end of the duct grows backward free from adjacent tissue, the cellular material being wholly derived from an anterior proliferation. For *Selachii* this method of origin has been maintained by Balfour ('78), and for *Amniotes* by a large number of observers; e. g. Weldon ('83) and Mihalkovics ('85) in *Reptiles*; Gasser ('77), Sedgwick ('81), Schmiegelow ('81 and '82), and Janošik ('85), in *Birds*; Renson ('83) and Martin ('88), in *Mammals*. Gasser ('82) believes that the segmental duct in *Alytes* has no direct connection with the mesoderm, posterior to the pronephros; but he was unable to exclude with certainty the possibility that the somatopleure immediately behind the pronephros might take some part in the formation of the duct. Mollier ('90, p. 226) moreover asserts that such a participation actually takes place in the two somites following those in which the pronephros is formed, but that the posterior portion of the duct probably grows back from this point independently of the mesoderm.

In so far as these authors maintain that the duct arises from a solid proliferation of mesoderm and acquires its lumen secondarily, I entirely agree with them; but my observations on this point lead me to conclude further that the duct arises throughout its entire length from a continuous thickening of somatopleure, and that the only free growth which occurs in the *Amphibia* studied by me is for the purpose of effecting a union with the cloaca. In assuming this position, I am aware of being in conflict with prior observations on *Amphibia*, and with the more recent accounts of the development in other groups; it seems to me, however, that satisfactory evidence in favor of this mode of origin has been adduced in the descriptive part of this paper.

Finally it remains for me to consider the third view, that of the ectodermal origin of the duct, which is to-day advocated on so many sides. As early as 1855 Remak expressed himself as dissatisfied with the derivation of the excretory system from the mesoderm, although this mode of origin was confirmed by his own observations. A decennium later His ('65^b, pp. 160-162) maintained that, in the Chick, the Wolffian and Müllerian ducts both arise as folds of the ectoderm; but he abandoned this position later ('68, p. 119), when it had been shown by Bornhaupt ('67) and Dursy ('67) to be untenable. He then endeavored to interpret the facts in harmony with his theoretical conceptions by maintaining that the cells from which the Wolffian and Müllerian ducts arose were primarily derived from the ectoderm, a view which was likewise adopted by Waldeyer ('70). Meantime Hensen ('66) had indorsed the view of a direct origin from the ectoderm. He states ('66, p. 81, foot-note) that

in the Rabbit the Wolffian duct arises from a solid rod-like thickening of the ectoderm near the middle protovertebræ. In a second short communication ('67, p. 502), Hensen merely reaffirmed his confirmation of His; but finally he ('75-'76, pp. 369-372) published a fuller account of his observations, accompanied with figures. These, however, are far from conclusive, and it does not seem surprising that this single observation was distrusted by subsequent writers.

In 1884 Graf Spee published an account of his very careful investigation of the subject, and reasserted the ectodermal origin of the Wolffian duct.¹ Following this publication have appeared a large number of confirmatory papers, which have moreover extended the observations of Graf Spee; so that at present the ectodermal origin of the duct has been asserted for every class of Vertebrates, with the single exception of the little known Dipnoi.

As stated in the Introduction to the present paper, it was my hope in undertaking these studies to find in Amphibia results confirmatory of Graf Spee's position. If, then, a contrary result has been reached, it has been because I have been driven to that conclusion by evidence brought out in the course of the investigation. In my opinion, the entire excretory system of the forms I have studied unquestionably develops without any participation of the ectoderm in its formation. The duct develops from mesoderm throughout its entire length, and at its posterior end, in *Rana* and *Bufo* at least, comes in contact with one of the entodermal cornua of the hind gut; so that *nowhere* in its development does it come into organic union with the outer germ layer.

I must in this case distinctly disavow the suggestion of Hertwig ('88, p. 280), who endeavors to harmonize the accounts by assuming that only the posterior end of the duct is formed from the ectoderm. This explanation would by no means be admissible, unless it be granted that the ectodermal constituent might in this case be reduced to nothing at all. On the other hand, it must be confessed that a fundamental opposition in the mode of development of an organ in two closely related groups is at present hardly reconcilable with our general conceptions of embryological processes.

¹ Graf Spee, and subsequently Flemming ('86), did not clearly recognize the fact that the Wolffian duct and the mesonephros develop in different ways, and were led to defend an ectodermal origin for the *excretory system*. This interpretation is in evident opposition to the accounts of others, and, in my opinion, is not justified by their own observation, even should these prove to be accurate in every particular.

It will therefore be of interest to review critically the most recent accounts in the several groups, for the purpose of ascertaining whether the ectodermal origin of the segmental duct be in any case actually demonstrated. For this purpose, only those papers which have appeared since Graf Spee's researches need concern us. Of these, the larger number are brief notices, which, in view of the extreme difficulty of the investigation, cannot be regarded as conclusive.

In regard to Cyclostomes, the only papers that have appeared during this period have been preliminary notices; that of Kupffer ('88) maintains an ectodermal, those of Goette ('88) and Owsjannikow ('89) a mesodermal, origin for the duct.

In Teleosts, the duct has been claimed to be ectodermal by Brook ('87) and Ryder ('87); but on the basis of my own observations, which are as yet incomplete, I am led to doubt the correctness of this claim, which has already been opposed by the observations of Hennequy ('88), of H. V. Wilson ('90), and of McIntosh and Prince ('88). In the account by Brook, it seems to me probable that the ectodermal thickening observed has in reality a very different significance (lateral line proliferation) from that attributed to it, an opinion which is shared by Wilson ('90, p. 58). The only recent paper dealing with the development of the Ganoidean excretory system is the preliminary notice of Beard ('89) on Lepidosteus. According to Beard, the duct is ectodermal.

In Amphibia, also, an ectodermal origin of the segmental duct has been asserted by Perenyi ('87) and by Brook ('87). Their communications, however, are both short notices, and in the absence of the final papers cannot be regarded as satisfactory evidence. Moreover, the mesodermal origin of the duct has been reaffirmed by Mollier ('90), Kellogg ('90), and Marshall and Bles ('90).

It is rather remarkable, that, in all the preceding classes, nothing but preliminary notices have ever appeared in favor of the ectodermal view. The same is true of Birds, where this mode of origin has been claimed as probable by Beard ('87) and by Brook ('87). On the other hand, a number of observers have carefully investigated the chick with this special purpose in view, and have been unable to find any evidence of a participation of the ectoderm in the formation of the Wolffian duct. Among these may be mentioned Janosik ('85), Mihalkovics ('85), and Hoffmann ('89). Peculiarly significant, however, is the fact that Graf Spee ('86) was unable with the use of the most various reagents to see any direct evidence of a genetic connection between the ectoderm and the Wolffian duct in the *Chick*.

In Reptiles, a number of writers have asserted that the Wolffian duct arises from the ectoderm. According to Perenyi ('87, '88, '89), irregular groups of cells are at an early stage budded off from the ectoderm covering the middle plate, and on the first formation of the segmental vesicles they form the cord of cells which has been recognized by prior writers as the fundament of the duct. In my opinion, no conclusive evidence is adduced to prove that the cells figured in the latter position ('89, Fig. 5, *ceW.*) are descendants of those which at an early stage form part of the ectodermal thickening. Mitsukuri ('88) and Orr ('87) have published short notes claiming an ectodermal origin for the duct; and, finally, Ostroumoff ('88^a, '88^b) asserts that it is derived from the ectoderm in *Phrynocephalus*. It seems to me, however, that Ostroumoff's observations are incomplete at a critical point, and that no satisfactory evidence is brought forward to show that the ectodermal thickenings which he describes and figures ('88^b, Tab. III. Fig. 56) with all desirable clearness, are unquestionably the fundament of the Wolffian duct. They may be merely chance thickenings over the intersegmental depressions in the underlying mesoderm. On the other hand, Mihalkovics ('85), Strahl ('86), and Hoffmann ('89) have all sought in vain to find satisfactory evidence of a participation of the ectoderm in the formation of the Wolffian duct.

With all the preceding classes of Vertebrates, I am of opinion that the weight of evidence is at present in favor of the view that the excretory system is wholly derived from the mesoderm. For the remaining groups, Mammals and Selachians, however, no such claim can be sustained. The researches of Graf Spee on *Cavia* showed conclusively that a cord of cells representing the fundament of the Wolffian duct is continuous posteriorly with a ridge of tissue which is still in intimate union with the superficial ectoderm, and that, in the further development, a continuous cord of cells separates off from this ridge by the progressive formation of a split between the deep portion of the ridge and the superficial ectoderm. At first, a distinct *membrana prima* is reflected from the unmodified ectoderm over the ridge, and the partially separated fundament of the duct may still be in connection with the superficial ectoderm by means of such a membrane. This latter feature is also dwelt upon by Flemming ('86), who furthermore emphasizes the circumstance that in the ridge which forms the first rudiment of the Wolffian duct mitoses are especially abundant, and that the nuclear spindles are frequently perpendicular to the surface, i. e. are so situated that the ensuing cell divisions would tend to thicken the layer. The

general results of these two investigators have been confirmed by Bonnet ('87 and '88) in the Dog and Sheep, and a number of former advocates of a mesodermal origin have satisfied themselves of the correctness of the opposed view by a study of the preparations of these authors; e. g. His (see Spee, '84, p. 93), Waldeyer (see Janošik, '85, p. 13), and Mihalkovics (see van Wijhe, '89, p. 501).

The most recent paper on this subject is that of H. Meyer ('90), who claims an ectodermal origin for the Wolffian duct in man. The embryo upon which these observations were made was obtained by artificial abortion, and was at once preserved by histological methods; so that, in the opinion of the author, it would be unfair to ascribe his results to imperfect preservation, which so frequently renders observations on human material untrustworthy. On the other hand, the mode in which the duct is here claimed to originate, viz. as a conspicuous *fold* of ectoderm, is so different from the method of origin described in other Mammals that one cannot regard this observation based on a single specimen as conclusive evidence.¹

A few recent writers have reasserted the mesodermal origin of the Wolffian duct even in the case of Mammals. Lockwood ('87, p. 642) criticises the evidence adduced by Graf Spee and Flemming, and compares their ectodermal ridge to a number of insignificant ectodermal thickenings which may be observed over depressions in the underlying tissue in diverse regions of the body. Lockwood entirely ignores the very definite relations which Graf Spee showed to exist at a certain stage between the fundament of the duct present in anterior regions and the continuous posterior ridge; his entire criticism therefore seems to me quite unwarranted. Fleischmann ('87) also reasserts in a preliminary note the mesodermal origin of the duct in Carnivora; but his description of the mode of origin is so entirely at variance with the accounts of recent authorities that his statements can hardly be regarded satisfactory before the evidence on which they are based is produced.

On the other hand, Martin (Stahl und Martin, '86, Martin, '88) accepts the main features of the development as described by Graf Spee and

¹ During the correction of these proof-sheets another paper has appeared which asserts a participation of the ectoderm in the formation of the duct in Man (Kollmann, '91). In the region of the middle plate there is found, according to this author, a close fold of ectoderm (Taf. III. Figs. 3, 4, *Anlage d. Urniere*, Fig. 8*) which he believes to be concerned in the formation of the duct, thus confirming Meyer's ('90) account. The later stages studied by Kollmann, however, are too far advanced to afford convincing evidence that his interpretation of the fate of this fold is accurate.

Flemming, but interprets their observations in a fundamentally different way. In the course of a painstaking investigation, in which more than forty series of sections were used, Martin never encountered conditions which in his opinion demonstrated a genetic connection of the duct with the ectoderm. He believes that the duct arises from a proliferation of mesoderm in the region between the 9th and 11th protovertebræ, and grows backward by cell division within its own mass. The posterior portion of the duct, however, fuses with the ectoderm so intimately that in certain regions it is quite impossible to recognize a boundary between them; but Martin believes that the fusion is wholly secondary, and that the ectoderm contributes no material to the duct. Keibel's ('88^a, p. 635) studies on *Erinaceus* led him at first to accept Martin's attempt at harmonizing the two views; but in *Cavia* ('88^b, pp. 424-428) his observations inclined him towards the original view of Graf Spee.

In my opinion, Martin is right in denying that an ectodermal origin of the Wolffian duct has been demonstrated in Mammals. It is undoubtedly true, that there is considerable evidence in favor of such a mode of origin; but it is not of a nature that would warrant one in concluding that the duct arises in this way throughout all Vertebrates, or in asserting that it develops in fundamentally different ways in Mammals on the one hand, and in other Vertebrates on the other. All that can be claimed, however, in accordance with Martin's view, is that it is possible to interpret the conditions in Mammals in agreement with observations in other Vertebrates, should these be shown to be less ambiguous.

In Selachians, the evidence in favor of an ectodermal origin of the duct is perhaps even stronger than in Mammals. In the former group, besides the preliminary communications of van Wijhe ('86, '88^a) and Beard ('87), there have appeared two extensive papers by Rückert ('88) and van Wijhe ('89), which seem to place the ectodermal origin of the segmental duct almost beyond question; and, so far as I am aware, no recent observer has expressed doubts upon this point. It nevertheless seems to me that, before accepting this result as final, we have yet to inquire whether Martin's interpretation of the condition in Mammals cannot be applied also in Selachii.

It might be objected, that the latter view offers no explanation for the intimate fusion which must be granted to exist between the posterior end of the segmental duct and the ectoderm; yet this argument cannot invalidate the general conclusion, since a number of cases of such a union of two epithelial structures in their growth have been recorded, where no genetic connection is believed to exist. Such a conception is in-

volved, for example, in the account — contested, it is true — given by Carius ('88) of the anterior growth of the chorda in the "Kopffortsatz" of *Cavia*, the chorda being in intimate fusion with the underlying ectoderm; or, again, the backward growth of the Amniotic Müllerian duct in close connection both with the Wolffian duct and with the adjacent peritoneum, as described by a number of recent writers.

In conclusion, then, I am of opinion that the more primitive condition, and that shown by most Vertebrates, is the development of the segmental duct independent of connection with the ectoderm; but that in certain groups the duct enters into a secondary union with the ectoderm. The question whether the ectoderm here contributes material to the fundament of the duct can at present receive no more definite answer than that contained in the foregoing discussion.

It has frequently been asserted that the mesodermal origin of the kidneys is not in harmony with our conceptions of the derivatives of this germ layer. As early as 1855 Remak saw a fundamental opposition in the mode of development which he described for the excretory organs, and that familiar in the case of other glands. According to his view, which received very general acceptance, the kidney is a unique example of a gland whose secreting surfaces are not derived from one or other of the bounding germ layers, ectoderm and entoderm.

In my opinion, this view must now be considered inaccurate. It is doubtless true that glands are usually developed either from the ectoderm or from the entoderm; this circumstance may merely be due to their apparently being seldom needed on mesodermal surfaces. Certain special regions, however, seem to require glands. Such regions are the sexual conduits in which, besides those glands which have special functions, such as the deposition of the secondary egg membranes¹ (Ludwig), we should expect to find glands similar to those which are found in the course of other canals leading to the exterior, such, e. g., as the trachea. I shall disregard the glands which develop in the ampullæ of the vasa deferentia, since these are derived from the Wolffian duct and consequently *may* be of ectodermal origin in Mammals, and shall take as a specific example the genital tract of the human female. It seems very certain that in Amniotes the Müllerian duct develops entirely independently of the Wolffian duct, as an evagination of the peritoneal covering of the Wolffian body. Moreover, whether we accept the view of van Ackeren ('89), that the hymen marks the region of fusion between the fused Müllerian ducts and

¹ The albumen secretion of the Hen's oviduct is a familiar example. According to Giacosa ('72) the oviducal secretion in *Rana* is largely composed of mucin.

the sinus urogenitalis, or that of Nagel ('89), who claims that the vagina is a product of the sinus urogenitalis, the boundary between the two constituents being marked by the os externum uteri, it must in either case be granted that the entire genital tract from the ostia abdominales of the oviducts to the os externum is of mesodermal origin. This entire system is lined with a continuous columnar epithelium, which is continuous below with the stratified epithelium of the portio vaginalis. In its histological characters this membrane closely resembles a typical mucous membrane, and is subject to the characteristic disorders of this form of tissue, cancer and catarrh. The Fallopian tubes are believed to be without glands;¹ in the region of the fundus and corpus, however, are numerous long tubular cœcæ which have been called uterine glands. It has not been demonstrated, however, that these structures exercise a secretory function; and they may merely serve to regenerate the mucosa cast in menstruation. In the cervical region occur glands (*glandulæ Nabothi*, *Schleimkrypten*) which are much shorter than those in the body of the uterus. These cervical glands secrete a viscous fluid of the characteristic ropy consistency of mucus, which at periods mingles with the catamenial flow,² and, in certain stages of pregnancy, forms a complete plug in the cervical canal. This secretion forms a dense mass on addition of alcohol; it swells conspicuously when placed in water; it stains blue with hæmatoxylin, and pink with picro-carmin; and, finally, according to Overlach ('85) its formation is attended with the same fundamental changes in the protoplasm at the distal end of the secreting cell which are familiar in the case of ordinary mucous secretion.³ It is almost certain that the cervical glands produce true mucus. Not merely, then, does the mesoderm give rise to glands, but *it produces glands of the same nature as those found in mucous passages of ectodermal origin*.

A second view was that formulated by His ('65*), according to which

¹ The vagina also is stated by Veith ('89) to be normally glandless.

² Of interest in this connection are the observations of Artemjeff ('89), who describes mucous corpuscles as a constituent element of normal lochia.

³ Through the kindness of Dr. C. S. Minot, I have been able to try in addition a few simple chemical tests on the cervical secretions. The cervical plug from a uterus of three months' pregnancy examined by me, proved to be soluble in potassic, sodic, and calcic hydrates, and in sodic carbonate; it is precipitated by nitric acid, but redissolves in excess; in strong acetic acid, on the contrary, it appears not to redissolve. The substance gives the proteid reaction with nitric acid, but not that with cupric sulphate. It also gave the specific mucin stain with methylen blue recommended by Hoyer ('90).

the ectoderm and entoderm alone are capable of giving rise to epithelial tissues. This view, which was associated with the derivation of the urogenital tract from the ectoderm, was naturally revived by Graf Spee ('84). More recent evidence, however, shows that it is only the Wolffian duct in regard to which the question of an ectodermal origin remains open; the Wolffian tubules, on the other hand, as well as the epithelia of the female sexual tract, are distinctly mesodermal. The statement that epithelia do not arise from the mesoderm is, in my opinion, either insignificant or untrue. If, avoiding genetic characters, we define epithelium so narrowly as to exclude endothelium, we must confess that, except in certain specialized regions, epithelia do not develop from the mesoderm; but the conclusion is obviously of little morphological importance. On the other hand, if we employ broad morphological characters in our definition, such a conclusion is manifestly inaccurate.

The ectodermal origin of the Wolffian duct has been supposed to account for certain pathological new formations which frequently have their seat in the urogenital organs. Thus His ('65^b) saw in the mode of development which he described for the Wolffian and Müllerian ducts an explanation for the occurrence of dermoid cysts in the ovary. It must be confessed that the structure of many of these cysts suggests that they have an ectodermal origin; but their occurrence in very diverse parts of the body shows that they do not require a normal ingrowth of ectodermal cells into the region in which they arise. Thus in the dermoid cysts which are occasionally found back of the optic bulb, the translocation must be regarded as purely adventitious.¹

The suggestion has recently been made by Sutton ('86, p. 344), that testicular and ovarian carcinomata are to be explained by the occurrence of degenerating ducts in the neighborhood of the genital ridge, and he is inclined to regard the Wolffian duct as the means of transporting ectodermal cells to this region. The weight of evidence seems to favor the view that carcinomata cannot develop without an epithelial basis (Klebs, '89, p. 771); but this fact does not compel us to seek an ectodermal source for these growths. In the case of adenomata, which also require an epithelial basis, one can see more readily the source of the proliferation; and these abound in the ovary. The germinal epithelium, in consequence of its retention of embryonic characters, seems to be well adapted to the formation of carcinomata, and, according to Birch-Hirschfeld's ('89, p. 202) enumeration, they are somewhat more frequent in the

¹ Many dermoids may be explained as cases of *fetus in fetu*, and those in the ovary may often be due to extra-uterine gestation.

ovary than in the testis, even though the latter organ is in such intimate relations with degenerating Wolffian canals.

The remaining portion of the present paper will be concerned with those inferences of a general nature which can be drawn from the development of the pronephros and segmental duct as traced in the preceding pages. These general conclusions naturally fall into two groups: (1) such as are of principal interest in elucidating the development of the excretory system, and (2) such as tend to throw light on the development of the Vertebrate type. Following this division, then, in our discussion, I shall consider in the present section the organogenetic conclusions; and, in concluding, deal with the phylogenetic conclusions which seem to me warranted by our present knowledge.

In the historical review of the development of the pronephros, it proved in several groups very difficult to draw a sharp line between the pronephros and the mesonephros, and it was suggested at that point in the discussion that this difficulty is in reality a fundamental one, and one which is indicative of the true relations between these parts. The question of the serial homology of the pronephros and mesonephros, as it presents itself to the modern student, is to my mind simply this: Are we to regard these two glands as derivatives of one continuous ancestral organ, which at one time extended over all the somites now occupied by each? The answer to this question naturally must come, if at all, by a comparison of the two organs for the purpose of bringing to light their features of similarity and those of contrast. Manifestly they differ in the time of their appearance; indeed, from this circumstance the two glands were distinguished and named; it remains to consider whether they are constructed on the same or on different types.

In endeavoring to furnish an answer to this question, I shall proceed to an anatomical comparison of the glands, taking into consideration both of the principal portions involved, the glomerular and the tubular parts. The glomerulus of the mesonephros resembles the glomus of the pronephros in the following particulars: both are highly vascular structures composed of ramifying blood-vessels and mesenchyme; they project into spaces which are in communication with the exterior by means of excretory conduits; they originate outside of this space, and gain position within it by pushing before them in their growth its epithelial wall, which then persists as an outer covering to the vascular process; they receive branches directly from the aorta; and, finally, they are developed in regions of the body which at least nearly correspond to each other serially,

as is shown by the relations of the glomms and the glomeruli respectively to the aorta, and by the existence of transitional glomeruli (Birds, Crocodilia, Chelonia). On the other hand, the features in which the glomus differs from the glomerulus may be summarized as follows: the glomus lies in the body cavity, instead of projecting into the lumen of a specialized excretory tubule, and it is a continuous structure, instead of consisting of a number of separate parts.

Turning now to the tubular portions of the two glands, one can recognize a number of common characters. In both can be distinguished a longitudinal conduit and transverse canals, the latter communicating with the body cavity by means of ciliated nephrostomes. The longitudinal canal of the two glands is in reality a continuous structure, the segmental duct. Since the pronephric and the mesonephric tubules are similarly related to this continuous duct, it is evident that they must themselves lie in approximately equivalent regions of the body. The metamerism of both glands primitively corresponds to that of the body somites; this feature is apparent from my account of the Amphibian pronephros, and has been proved for the most anterior mesonephric tubules in *Amblystoma* (see page 261), as well as for the entire series in *Selachii* and certain other groups. Finally, the cardinal veins give rise to a meshwork of vascular spaces which bathe in a like manner the tubules of the pronephros and mesonephros. In addition to the different ways in which their tubules are related to glomerular structures, the pronephros and mesonephros are unlike, in that the tubules of the former develop in continuity with the duct, while those of the latter join the duct secondarily. The character of the convolution also is different in the two glands. As is evident from the reconstructions (Plates IV. and VIII.) of the pronephros in *Rana* and *Amblystoma*, the complication is here mainly due to the convolution of the longitudinal canal (common trunk); whereas in the case of the mesonephros the longitudinal canal (segmental duct) traverses the gland as an almost straight duct, the transverse tubules alone being highly convoluted.

The pronephros and mesonephros, then, present many striking anatomical features of resemblance, but also differ in several respects. I am however of opinion, that the similarities of structure are sufficiently great to make it probable that pronephros and mesonephros have developed from a common beginning. I do not think, however, that such tabulation of the resemblances and differences gives an adequate insight into the true relationships of the structures. In the search for ancestral characters, it is a matter of indifference whether the organ in ques-

tion actually realizes a given character, or merely shows a tendency to assume it, provided in the latter case it can be satisfactorily shown that the realization of the tendency was prevented by intelligible causes. Thus, in the gastrulation of meroblastic eggs, if it be recognized that the great accumulation of yolk renders emboly impossible, the substitution of epiboly in these cases must be regarded as morphologically insignificant.

The question now naturally arises, Are any of the contrasts between pronephros and mesonephros of such a nature that they can be explained as the result of a single modifying influence? As I have already stated, the most marked point of disagreement between the two glands is the difference in time at which they appear. What influences may that factor exert in modifying their development? At the time when the Amphibian mesonephros appears, the myotomes are widely separated from the peritoneum, and the continuous strip of cœlom immediately ventral to the lower boundaries of the protovertebræ in the region of the pronephros does not exist in the region of the body in which the mesonephros develops. In its place is a mass of cells which extends from the dorsal angle of the body cavity upward towards the overlying myotomes. This mass of cells has been regarded as the first rudiment of the mesonephros. The most natural explanation of the condition is that this mass of cells is morphologically not a secondary proliferation from the peritoneum, but is really the last remnant of the mesoderm which formerly connected the dorsal angle of the permanent body cavity with the overlying protovertebræ. The correctness of this interpretation is shown by comparison with the conditions in Selachians and in Amniotes, where, according to the mutually confirmatory accounts of Sedgwick ('80*), Van Wijhe ('88*, '89), Rückert ('88), and Hoffmann ('89), the mesonephric tubules develop from the communicating canal. The first rudiment of each mesonephric tubule is in reality that portion of the primitive mesodermal plate which lies immediately ventral to the protovertebræ, and, corresponds to that portion of the cœlom into which, as shown in Figure 6, the glomus projects, and from which the pronephric tubules emerge. *Each mesonephric fundamen*t, then, *presents on its outer side somatic, on its inner, splanchnic mesoderm.* When the fundaments of the mesonephros have been converted into a series of blind tubules, they grow outward and join the segmental duct. This process appears to me to be precisely equivalent to the *somatopleural* evagination, which at an early period gave rise in the anterior region to the nephrostomal tubules of the pronephros. That portion of the differentiated mesonephric tubule into which the

glomerulus projects is of different origin ; it is merely a portion of the coelom, the walls of which are to be understood to be formed as I have just stated in part by somatic, in part by splanchnic mesoderm.

Returning now to the two features in which the glomus was shown to differ from the glomeruli,—viz. situation within the body cavity, and continuity throughout successive somites,—it will be seen that it is impossible to maintain the former as a ground of distinction, since the glomerulus also lies in a detached portion of the coelom, and that the latter ground is equally untenable because it simply results from the fact that, before the glomeruli appear, the space into which they would otherwise project as a continuous organ has already been broken up into a series of distinct tubes ; the glomerular organ is consequently broken up into a corresponding number of separate vascular processes, each of which becomes converted into a Malpighian capsule.

It seems probable, therefore, (1) that the pronephros and mesonephros were primitively alike, and were portions of a single continuous gland ; (2) that in Vertebrates which came to lead an independent existence early in life, an anterior portion of the gland and the whole of the duct are differentiated before the posterior part for the immediate purposes of the larva ; and (3) that the difference in structure between the two glands is mainly due to their arising at different times relatively to the differentiation of the body cavity and protovertebræ. Applying this conclusion to the tubular portion of the glands, it becomes at once intelligible why the tubules of the mesonephros must of necessity join the duct secondarily. From this standpoint, the existence of convolutions in the common trunk points to a less differentiated condition of the pronephros, in that, for temporary purposes, the longitudinal canal, including the common trunk, subserves at the same time the functions of an efferent duct and of a secreting tubule.

The foregoing explanation of the nature of the pronephros is based upon the assumption that it is developed as a larval excretory organ. In order to justify this position, it will be necessary to consider whether the pronephros is functional in those Vertebrates which, viewed from this standpoint, would seem to require this organ, and in such alone. For the present purpose, two methods of sexual reproduction may be distinguished : (1) that in which the mother spends her energy in producing a large number of offspring, which are early forced to care for themselves ; and (2) that in which the mother produces a small number of eggs, and, either by giving to each a large quantity of reserve food yolk, or by nourishing the young embryo within her own body, secures the existence

of her offspring without calling into play their individual activities. In the former class may be reckoned Cyclostomes, Teleosts, Ganoids, Dipnoi, and Amphibia.¹ Omitting from consideration the little known Dipnoi, a functional pronephros appears in all the members of this group without exception, and is most highly developed in those forms (Petromyzon, Amphibia) which pass through a protracted larval stage. The other class includes Selachians, Reptiles, Birds, and Mammals. In every member of this group the pronephros is rudimentary.

I conclude, therefore, that pronephros and mesonephros are parts of one ancestral organ; that the glomeruli are strictly homodynamous with the glomus; that the entire tubular portion of the pronephros is represented in the mesonephros; that the cavity of a Malpighian capsule and the nephrostomal canal connecting it with the body cavity are detached portions of the coelom, the equivalents of which are not thus differentiated in the pronephros; that the pronephros is developed as a larval excretory organ; and that the period at which it appears largely accounts for its peculiarities of structure. This general conclusion, which is mainly based upon a study of the conditions in Amphibia, is, in my opinion, in perfect harmony with the recorded observations on other groups.

It must be remembered in this connection, however, that the pronephros may possibly have been developed from the primitive excretory organ independently in two or more groups, in response to similar physiological necessities. While I have not been able to preclude this possibility, I am nevertheless inclined to the opinion that in general a closer relation exists, and that consequently the pronephros is homologous throughout all Vertebrates. An interesting condition manifests itself in those forms (Teleosts and Ganoids) in which the pronephros remains functional until the individual is nearly adult. In these the pronephric chamber becomes partially (Lepidosteus) or entirely (Teleosts) cut off from the body cavity and comes to resemble an enormous Malpighian capsule. The region in Crocodilus intermediate between pronephros and mesonephros shows a

¹ In the one group, the eggs are holoblastic, or if meroblastic contain little yolk (Teleosts); in the other, they contain much yolk, or the young are nourished by means of a placenta (Mammals). Mr. Samuel Garman has kindly called my attention to a number of cases in Amphibia where the period of larval life is greatly reduced. The occurrence of holoblastic segmentation in this group appears to me to afford adequate evidence that such conditions are secondary. Moreover, there actually appears to be a reduction of the pronephros in such species as abandon in part their larval life. In the case of *Hylodes martinicensis*, mentioned by Mr. Garman in this connection, Selenka ('82) has shown the pronephros to be very degenerate.

similar differentiation of a part of the coelom into a distinct excretory chamber. The condition in this region differs from that of the mesonephros of this genus solely in the circumstance that the excretory chamber is not broken up into metameric portions; this process takes place in the posterior region, and produces a typical mesonephros.

It now remains for me to review the opinions of previous writers in respect to the nature of the pronephros. The existence of a larval excretory system different from and earlier than the mesonephros appears to have been first suggested by Marcussen ('51); but this view received no recognition until it had been reasserted by Wilh. Müller ('75), who gave to the pronephros a distinctive name, *Vorniere*. Semper ('75), on the other hand, denied utterly the nephridial nature of the pronephros, and regarded the glomus as equivalent to the suprarenals (Nebennieren) of Plagiostomes. Fürbringer ('78^a) vigorously opposed this view, and maintained that the pronephros and its duct represent a primitive excretory system which conspicuously differs from both mesonephros and metanephros. According to Balfour's ('75) earlier view the segmental duct is formed by the backward growth of a single anterior evagination, which may be regarded as the representative of a mesonephric tubule. He ('81) later interpreted the pronephros similarly to Fürbringer, but was still inclined to believe that each mesonephric tubule was "in a sort of way serially homologous with the primitive pronephros." It is very difficult for me to reconcile the latter opinion with his view that the pronephros is a primitive excretory system derived from Plathelminthes, while the mesonephros is a secondary (new) development which does not appear until the trunk becomes segmented. Moreover, this view manifestly ignores the metamerism which is exhibited by the pronephros. It appears to me therefore entirely unsatisfactory.

Sedgwick ('81) first distinctly stated the conclusion that the pronephros and mesonephros are differentiations of a single ancestral organ. This view, which was adopted by Renson ('83), does not seem to have been generally accepted, although several authors, by describing what they denominate a transitional region, seem to me implicitly to assume an intimate connection between the two glands. Mihalkovics ('85, pp. 65, 66) denied that they are *wholly* homologous, on the ground that the pronephric tubules are peritoneal evaginations, whereas those of the mesonephros are differentiated in the solid Wolffian blastema. Mihalkovics does not explain *his* use of the term *complete* homology, and I have been unable to satisfy myself in regard to the precise relations which he supposed to exist between the two glands.

Van Wijhe ('88^a, '89), Rückert ('88), Hoffmann ('89), and Wieder-
sheim ('90), have distinctly denied the serial homology of the pronephros
and the mesonephros. The objections of these authors to the view which
I have adopted have been most clearly formulated by van Wijhe ('89,
pp. 509, 510), whose account I shall follow in my criticism of their
position. First, "the pronephros arises before the appearance of the
duct or the mesonephros, and is indeed the first part of the excretory
system that appears." This point of difference is, as I have stated, the
most conspicuous feature in which the two glands are unlike. It is, how-
ever, not a weighty argument against their serial homology. Secondly,
"the pronephros arises as an (in *Selachii* segmented) evagination of the
somatopleure; its cavity, which may be temporarily obliterated by the
proliferation of the walls, is formed as an evagination of the body cavity
(Metacölon). The mesonephros, on the other hand, is not formed as an
evagination, and it is constituted of somatopleure as well as of splanchno-
pleure." This analysis seems at first sight to establish a fundamental
contrast between the pronephros and the mesonephros, and I admit fully
the cogency of the argument in disproving a comparison of the nephro-
stomal and glomerular portions of a mesonephric tubule with the
nephrostomal canal of the pronephros. On the other hand, however, I
would insist that a hitherto unnoticed homologue of the pronephric evagi-
nations is to be found in the *outward growth* of the primitive mesonephric
canal to join the duct.¹ (See page 301.) It is in precisely this way that
a tendency to a somatopleural evagination would of necessity manifest
itself. Thirdly, "the duct always appears in continuity with the pro-
nephros, but always discontinuous with the mesonephros, which only
secondarily fuses with it and empties into it." This circumstance, as I
have already shown, is a direct consequence of the condition explained
under the first head. Fourthly, "the mesonephros possesses Malpighian
corpuscles; while the pronephros has none, the glomus of the latter not
being homodynamous with the glomeruli of the mesonephros because it
is a vascular tuft invaginated into the secondary body cavity (Meta-
cölon)." This contrast appears to me morphologically inaccurate, as I
believe I have adequately shown in the preceding discussion.

A further objection, which van Wijhe does not mention in his enumera-
tion, is the occurrence of rudimentary mesonephric tubules in the somites
which formerly gave rise to the pronephros. To prove this assertion, it

¹ This is the only portion of the mesonephric tubule which can properly be called
an evagination; the entire tubule comprises the evagination plus the communicat-
ing canal.

is usually regarded adequate to show the existence in the pronephric region of metameric diverticula proceeding from the body cavity towards the overlying protovertebrae. These diverticula are the communicating canals, and it is undoubtedly true that from similar canals in the posterior region mesonephric tubules are actually developed; but, to my mind, the occurrence of these diverticula in the pronephric region cannot be brought forward as evidence of the existence of two sets of nephridial tubules in these somites, until it can be shown that these remnants of the canal-like communication between protovertebrae and lateral plates exhibit some indication of the characteristic nephridial differentiation, i. e. grow outward and join the duct. This, I believe, has never been *demonstrated*. The existence of such a growth has, however, been asserted by several observers; but it seems to me compatible with the view I have expressed of the relations between pronephros and mesonephros. Since the time of the investigations of Balfour and of Semper on Selachians, it has been a familiar fact, that, although at first only one mesonephric tubule occurs in each somite, the further complication of the gland is largely produced by the formation of new tubules which proceed from the region of the primary Malpighian capsule. If the development of more than one tubule in a somite became normal in the ancestors of the Craniotes before the separation of pronephros and mesonephros took place, the development of such secondary tubules in the pronephric region would at once be intelligible.

A more fundamental objection is contained in an ignored observation of Gasser ('82, p. 96) on *Alytes*, according to which a typical glomus is developed in the body cavity of the mesonephric region, in addition to the universally present glomeruli. Gasser's account is contained in a rather short note unaccompanied by figures; it has not been confirmed by any subsequent observer; nor have I been able to find such a structure in either *Rana* or *Amblystoma*. I am therefore inclined to the opinion that Gasser may have mistaken for the glomus either the germinal ridge or the fat-body, both of which are developed in this region, although this explanation would contradict the statement of Gasser that the mesonephric glomus is a transitory organ. Be that as it may, I cannot without further evidence accept his account as final.

Semon ('90) has recently asserted that the pronephros and mesonephros are built upon the same structural type. He was led to this conclusion by a study of the excretory system in Ichthyophis. I have already alluded to the condition of the pronephros in this form. It is characterized by the possession of a completely closed pronephric cham-

ber, from which a portion of the nephrostomes ("inner" nephrostomes) emerge. Each nephrostomal canal, however near the nephrostomal end, is joined by a branch which communicates with the permanent body cavity by means of an "outer" nephrostome. According to Semon, the pronephric chamber, as well as the cavity of a mesonephric Malpighian capsule, is a diverticulum of the cœlom; and the nephrostomal canal which joins the glomerular portion of a mesonephric tubule with the body cavity is represented by those canals of the pronephros which emerge from the open body cavity. The mesonephros is to be regarded as a "generation" of excretory tubules younger than the pronephros, and the latter may be conceived to have primitively extended throughout the entire trunk. In many features Semon's view is similar to that expressed in the preceding pages. The point of difference which I would here emphasize is the different way in which the nephrostomal canal of the mesonephros is explained. According to my opinion, *this canal is a remnant of the communication between the protovertebral cavity and the secondary body cavity, and is not represented in the tubular portion of the pronephros.* Semon, on the other hand, claims that it is the homologue of the outer series of nephrostomal canals in the pronephros of Ichthyophis. Considering the relations of the glands in that form alone, this view seems well justified; but it neglects the significant relation which has recently been shown to exist between the mesonephros and the communicating canal; and I am of opinion that the view as applied to other Vertebrates is untenable, unless it can be shown that the outer nephrostomal tubule of the Gymnophionian pronephros also develops from that canal. The latter interpretation is, I must admit, at least possible; but we must await further researches on the development of these Amphibia before accepting such a conclusion.

The closing section of this discussion will be devoted to a consideration of the evidence which the development of the excretory system as a whole throws on the origin of Vertebrates.

Two methods of investigation, which are mutually dependent, yet quite unlike in their application, may be employed in attempting to draw phylogenetic conclusions. One of these methods is peculiar to embryological research; it is dependent upon the principle that ontogeny is in part an abbreviated recapitulation of phylogeny; its method is to eliminate cœnogenetic characters; it accomplishes this largely by the aid of a physiological estimate of the influences of larval and embryonic environment, and it is comparative only throughout

the extent of the group whose origin is sought. The other method is common both to comparative embryology and to comparative anatomy; it is dependent upon the inherent improbability of the same physiological requirements, being met by the same structural device in two groups which are not genetically related; it can employ equally well, though with a somewhat different significance, both cœnogenetic and palingenetic characters; it is purely anatomical in its method, and it is in the broadest sense comparative. The first I may designate as the method of elimination, or the intensive method; the latter as the comparative, or extensive method.

I have been led to make the preceding analysis in order to employ the division thus indicated in the subsequent discussion, and also because it is a contrast which does not appear to be generally recognized. Thus, a recent text-book of zoölogy (Hatschek, '88, pp. 25, 26) identifies the methods of embryology with those of comparative anatomy, and declares that palingenetic and cœnogenetic characters are equally valuable for phylogenetic inferences. According to the preceding analysis, these two statements are partial, relating only to the comparative method in embryology, and ignore the higher use which renders embryological facts of peculiar value.

Observing then this two-fold division in the following discussion, an attempt will first be made to reconstruct from the ontogeny of Vertebrates the ancestral history of their excretory organs.

The most general character which appears to be common to the ontogeny of all Vertebrates is the intimate relation which exists between the excretory tubules and the cœlom. This relation is peculiarly well illustrated by the pronephros, but it is true also of all the urogenital organs, and is a fact which in my opinion throws considerable light on their evolution. The cœlom appears to be an internal cavity developed to meet a number of physiological needs. It is likely that in the lower Invertebrates the cœlom served largely a nutritive function (see, e. g., Chun, '80, pp. 248-253); but I am of opinion that in the higher Invertebrates and in Vertebrates the cœlom early became in large measure an excretory space. This function of the cœlom, inferred from its relations with nephridia, is in accord with its situation in the body. Evidently the organs which would be most in need of a near place of discharge for nitrogenous waste products are those which are in the highest degree metabolic. Such are, *par excellence*, the muscle masses of the body, and it is a familiar circumstance that in all Chordates the primitive muscle plates develop from the lining wall of the dorsal segmented por-

tion of the cœlom. It is very probable that this arrangement represents the earliest differentiation of a special excretory surface of which evidence is preserved in the ontogeny of Vertebrates.

The next step in the specialization of the urinary organs is the establishment of definite conduits for the purpose of conveying the excreted products to the outside. It is possible that simple apertures, such as the abdominal pores, at first served this end; or, if the enterocœlous condition represent a phylogenetic stage, communications with the intestinal tube may have afforded an outlet to the excreted fluids. Be this as it may, it is evident that the ancestors of our present Vertebrates early acquired specialized tubes subserving this purpose.

In the account of the development of the Amphibian pronephros and duct given in the first section of the present paper, emphasis was laid upon the fact that these structures are differentiated from a solid somatopleural thickening, and do not arise as a fold of the peritoneum. Manifestly the former condition is cœnogenetic; such a solid thickening could in no wise function as an excretory conduit. On the other hand, it must not be rashly assumed that the somatopleural thickening is a disguised *fold* of that layer. On the contrary, the pronephros, on canalization, shows itself to be already composed of a series of metameric evaginations of the cœlom, and it is perfectly conceivable that the pronephric thickening is a modification from a condition where the separate evaginations had their *independent* means of communication with the exterior, the several diverticula being fused into a solid mass. Either interpretation would be physiologically intelligible. In the first case, a certain region of the peritoneum would first sink as a groove into the parietes of the cœlom. This channel might, like the nephrostomes, be provided with vibratile cilia, and might thus serve to carry the fluids lodged in it back to a single pair of orifices situated near the posterior end of the cœlom. As a further differentiation, it is to be conceived that this groove became at intervals constricted off from the cœlom, forming a retroperitoneal duct with a series of nephrostomal tubules.

According to the second alternative, it is necessary to suppose that the several evaginations communicated distally either directly with the exterior or with an independent longitudinal duct. The nephridia of *Heteromastus* and *Capitella* (Eisig, '88, pp. 242, 272), in which no external opening is present, show us that the gradational steps in the formation of such outgrowths may be conceived to be functional.

In judging between the two views to which allusion has just been made, it is important to consider whether the ontogeny of other groups

ever presents either of these processes in an unambiguous manner. I have already expressed my doubts in regard to the development of the pronephros and duct by the incomplete closure of a groove of somatopleure. The best attested claim that has been made for such a mode of origin was that made by Goette, Fürbringer, Hoffmann, and Marshall and Bles, for Amphibia; but this position is distinctly contradicted by my own observations. Indeed, this mode of origin has been recently denied in the case of every class except Teleosts, a group in which it is very difficult to obtain accurate evidence respecting the early history of the mesoderm.

On the other hand, numerous recent investigators have described the first rudiment of the pronephros as a series of distinct evaginations. Such observations have been recorded in Cyclostomes by Kupffer ('88), in Ganoids by Beard ('89), and in Anniotes by almost all writers on their early development. It seems to me, therefore, that the mode of formation by means of serial evaginations has a far wider distribution, and is more clearly attested, than that by means of an incompletely closed fold. I am of opinion that the condition in Amphibia and Selachia is to be regarded as derived from such evaginations by means of cœnogenetic modification; and that the weight of internal evidence is in favor of the view that the tubules were primitively distinct.

Typically the nephridial tubes are strictly metamerie, one pair of tubules being developed in each metamere. The occurrence of several nephridia in a somite occurs, as we have seen, in the case of the mesonephros of certain Amphibia. This condition seems to me to be a character secondarily acquired. The following reasons confirm this opinion: (1.) In other forms, the strict metamerism of the nephridia is the earliest ontogenetic condition, the duplication of the tubules appearing much later. (2.) The dysmetamerie arrangement seems to be correlated with the limited number of somites which are, in such cases, involved in the formation of the mesonephros; thus, in the Anura, a group in which the number of trunk somites is extremely small, the mesonephros departs most widely from the metamerie condition; in Urodeles, the number of somites is larger, and there is an indication of metamerism in the anterior tubules; and again in Cœcilia, where the number of somites is still larger, the mesonephros has the typical metamerie arrangement. (3.) The pronephros, which in general represents the least modified portion of the excretory system, retains a metamerie condition in those forms in which this arrangement is absent in the mesonephros.

In order to ascertain the probable mode in which the metamerie diver-

ticula primitively terminated, whether they opened on the surface or joined a longitudinal duct, it will be necessary to consider the pronephros alone, since the segmental duct is already present before the mesonephros is formed, and we cannot expect to find an adequate criterion for determining whether the union of the mesonephric tubes with the duct be primitive or secondary. In the pronephros there is in most cases no evidence of a mode of termination more primitive than that of communicating with a duct. Two arguments, however, occur to me, which seem to indicate that a series of direct outlets to the exterior may have been early present. In the first place, the pronephric diverticula have frequently been observed to enter into intimate union with the ectoderm. Thus Rückert ('88, p. 217) was led to believe that the pronephric thickening of Selachians even received a contribution of cells from the outer germ layer. The most natural explanation of this condition seems to me to be, that the fusion of the diverticula with the ectoderm is the recapitulation in the ontogeny of a phylogenetic stage, which possessed nephridia provided with direct openings to the exterior. Secondly, *Amphioxus*, according to the most recent investigations, is provided with a series of nephridia opening into the atrial chamber, which latter we are, in my opinion, justified in regarding as a simple infolded portion of the exterior. Accepting the homology of the nephridia of *Amphioxus* and those of Craniotes, it seems to me probable that the ancestors of Vertebrates possessed nephridia which resembled those of *Amphioxus* in opening directly to the exterior.

If separate diverticula leading from the cælom to the exterior be the primitive condition of the Vertebrate excretory organs, we have still to seek the origin of the segmental duct. On this point, the pronephros alone can afford evidence. The participation of the ectoderm maintained by many authors for the posterior end of the duct affords the suggestion that it may have first been formed as a groove of that layer, or that a primitive anterior opening was gradually shifted back to the cloaca. It may be objected to this view, (1) that in many Vertebrates no participation of the ectoderm occurs, while in none has it been shown that the mesoderm does not play a part in the formation of both anterior and posterior portions of the duct; and (2) that the longitudinal canal of the pronephros, which forms the anterior prolongation of the duct, in no case arises in this way. In the pronephros the longitudinal canal arises, as testified by a large number of recent investigators for divers groups, and as confirmed by my own observations on Amphibia, by the fusion of the distal ends of the pronephric diverticula. This mode of development

seems to me entirely in harmony with physiological requirements; and in this earliest fragment of the excretory system we have, in my opinion, a remnant of the primitive mode of formation of the segmental duct.

The question at once arises whether there is any indication of this mode of origin preserved in the development of the posterior portion of the duct. A free backward growth, such as is maintained for many Vertebrates, is evidently far removed from the primitive mode of formation, and is to be regarded as an adaptation to the needs of the pronephros. The origin of the duct *in situ* from a somatopleural proliferation is without doubt a modified condition; yet it suggests a mode of origin which is in agreement with that observed in the anterior region. I have already emphasized the circumstance that in Amphibia the duct arises from a mass of cells which is perfectly continuous with that from which the pronephric tubules are differentiated; and it is possible that both regions represent disguised nephridial evaginations of which those in the posterior region are never differentiated as actual canals except in such portions as are converted into the duct. Further evidence in favor of this view is afforded by the occasional occurrence of supernumerary pronephric tubules such as have been observed by Mollier ('90, p. 224) and myself (page 253). The acceptance of this interpretation would necessitate a modification of our conception of the relations between pronephros and mesonephros, since we should be obliged to regard the mesonephric tubules as a second generation of tubules, the first generation having been employed in giving rise to the duct. On the other hand, it is quite possible that the entire backward growth of the duct is a *wholly* secondary process to meet the needs of a prematurely developed portion of the primitive excretory organ. This is the only interpretation which seems admissible in those cases where the duct has been found to grow backward free from adjacent tissue.

The conception of the phylogeny of the duct which I have just presented offers a partial explanation of the contradictory evidence which has been advanced respecting the germ layer from which the duct arises. With a narrower conception of the phylogeny of the duct, it is difficult to understand why the ectoderm should participate in the formation of the excretory system in one group, but not in another, and why the posterior end of the duct should in some cases be formed at the expense of a germinal layer different from that which gives rise to its anterior portion and to the nephrostomal canals wherever they appear. If, however, we assume the existence of a phylogenetic stage in which a series of nephridia open directly to the exterior, it is at once evident that a

very trifling difference of location would determine whether the longitudinal canal, by means of which the duct arises, should develop from the mesoderm or from the ectoderm. It is to be remarked, however, that such an explanation is not wholly satisfactory, since one would expect on this hypothesis that those forms in which the ectodermal origin of the duct seems well attested would show evidence of close genetic relationship, while those classes in which the duct arises from the mesoderm ought to form an equally well defined group. This condition, however, is by no means realized. On the other hand, the force of this objection is materially weakened if we regard the duct as a recent acquisition, which its absence in *Amphioxus* gives some justification for assuming. The explanation seems to me, nevertheless, in a measure unsatisfactory, and I have adduced it merely as a possible solution of the problem to which the apparently diverse relations of the duct to the germ layers gives rise.

An intimate relation is always very early established between the excretory tubules and the cardinal veins. Such an arrangement is so favorable for the process of secretion that there can be but little doubt that this condition prevailed in the ancestors of all Vertebrates. There does not appear to be any evidence which would indicate whether the cardinal veins or the excretory tubules are the more primitive structures.

In addition to the means of excretion afforded by the epithelial walls of the tubules, the Vertebrate kidney-organs possess peculiar glomerular structures. These, as I have already shown, are all formed on the type of the pronephric glomus. In their primitive condition, they consist of vascular tufts, which receive blood from the aorta and project into the body cavity from the root of the mesentery.¹ The origin of such a primitive glomerular structure is not far to seek. It is readily conceivable that fluid may at first have simply exuded from the aorta, and, traversing the small amount of tissue intervening between it and the body cavity, may have reached the orifices of the excretory tubes prior to the development of any specialized organ subserving a glomerular function. This process being once established, any modification of structure which should allow a portion of the aortic current to be brought into closer relations with the excretory tubules would be of obvious utility, and would be preserved.

The excretory system thus constituted would represent the proneph-

¹ The view of the excretory system here presented explains the double blood supply of the kidneys of lower Vertebrates, and also the circumstance that the Malpighian bodies always receive their blood by a direct branch from the aorta.

ric type of structure. I have already sketched the manner in which the mesonephros may be derived from the pronephros by supposing the metameric segmentation of the body to extend to that portion of the coelom from which the nephrostomes emerge. The account given in the preceding section of this paper regarded the tubules as passive in such a metamorphosis. It is possible, however, that the transference of the tubules to a segmented portion of the coelom may have been in part effected by a dorsalward shifting of the nephrostomes. In either case, I am of opinion that the mode of development which I have now suggested is applicable alike to the pronephros and the mesonephros, and I may also add to the metanephros (see Sedgwick, '80).

I have now presented, in a suggestive manner rather than as a complete argument, certain indications of the phylogeny of the excretory system which may be obtained from internal evidence. It still remains for us to consider what conclusions are justified by a comparative study of the excretory system, and whether the phylogenetic stages suggested in the foregoing account are to be found in any group of living animals.

The sole purpose of this discussion is to ascertain the most probable phylogenetic line of development for the excretory system of Vertebrates. For this reason, I shall avoid any discussion, which would necessarily be lengthy, respecting the interrelationships of the diverse excretory organs found in Invertebrates, simply endeavoring to seek out those classes which possess nephridia similar to those of Vertebrates, and shall ignore the further consequences which would follow from the assumption of a homology in any single case.

In the preceding account, I have provisionally accepted the view that *Amphioxus* belongs to the Vertebrate phylum, and have endeavored to interpret its kidneys in accordance with that view. With Tunicates it is quite different; not merely do they afford no assistance in the solution of the problem in hand, but it has hitherto proved impossible to find any organs in this group which can be regarded as homologous to Vertebrate nephridia.¹ In my opinion, it cannot be objected that the absence of such organs in Tunicates proves that the Vertebrate nephridia arose within the Vertebrate phylum. A rigid adherence to such a system of restriction in the case of other organs would quickly lead to absurd conclusions.

The only classes of animals in which we need seek for a homology of

¹ Hatschek ('84, p. 519) regarded the single nephridium described by him in *Amphioxus* homologous with the neural gland of Tunicates; but I have already pointed out the probable inaccuracy of this observation.

the Vertebrate renal organs are those belonging to the bilateral clodus; and among these I shall consider only those forms which are usually included in the rather heterogeneous class Vermes. This restriction is justified by the circumstance that the only similarities of structure which are to be found between the excretory system of Vertebrates and those of Mollusks and Arthropods recur with greater force in the case of several groups of Vermes.

In comparing the kidneys of Vertebrates and those of Worms, I shall distinguish three types of structure in the latter group: (1) the water-vascular system of Plathelminthes, (2) the excretory system of Nemeritines, and (3) the nephridia of Annelids. The various organs which serve as excretory and genital passages in Rotifers, Nematodes, Echiurids, and Sipunculids are either referable to one of these types, or are valueless for the purpose in hand.

In endeavoring to find what points of similarity exist between the excretory system of Plathelminthes and that of Vertebrates, I have been unable to formulate any more definite statement than that both consist of longitudinal internal canals, which bear numerous lateral branches, and which open directly or indirectly to the exterior. On the contrary, the two sets of organs appear to me to perform the function of excretion by anatomical devices which are diametrically opposed. In Vertebrates, the excretions are either (primitively) poured into the *cœlom* and conveyed thence by a simple series of conduits, or excreted from the blood in the course of the *tubuli uriniferi* of the kidneys. In Plathelminthes, on the other hand, the excretory tubes ramify throughout the entire body parenchyme, and, so to speak, seek out the waste products of metabolism at the seat of their formation. It is a contrast such as exists between lungs and tracheæ, and appears to me of fundamental importance. According to Fraipont ('80) and Francotte ('81 and '83, pp. 734, 735), it is true, there is a communication between the excretory tubules of Plathelminthes and certain interior canalicular spaces, which they interpret as a rudimentary *cœlom*. The evidence in favor of the latter interpretation is certainly far from complete, but could not, if true, overthrow the fundamental contrast which I have just emphasized. Furthermore, I am not aware that any subsequent writers have confirmed this account of the termination of the excretory capillaries; while Pintner ('80, p. 302), von Graff ('82^a, pp. 106 *et seq.*, '82^b, p. 80), Lang ('81, p. 208, '84, p. 167), Iijima ('84, p. 400), Zschokke ('87, p. 165), and Böhmig ('90, p. 243) have all asserted that the terminal sacs are entirely closed. The conclusion seems warranted that no direct evi-

dence in support of an intimate relation between Vertebrates and Plathelminthes is afforded by a comparison of their excretory organs.

In Nemertines the excretory system is in peculiar relations with the blood vascular system. According to Oudemans ('85), the excretory system of the Schizonemertini and the Hoplomertini consists of a longitudinal tube, which is closely applied to the lateral longitudinal blood-vessel of the œsophageal region, and this tube communicates with the exterior by means of a single excretory pore or by a number of such openings. In Carinella and Carinoma, however, the connection is much more intimate, and the glandular portion of the excretory organ lies embedded in the œsophageal blood lacunæ and communicates with the latter by means of two or three evident openings. Oudemans asserts, indeed, that the excretory system is in reality a detached portion of the blood vascular system. Bürger ('90, p. 92), however, has recently thrown some doubt upon the existence of open communications between the nephridia and the blood-vessels, but reaffirms the close dependence of the excretory system upon the vascular trunks.

Comparing the nephridia of Nemertines with those of Vertebrates, it seems to me that one cannot fail to recognize a pronounced difference in type. In Vertebrates the nephridia are canals in close relation with the cœlom; they develop from its epithelium, and even in the adult arise from chambers which must be regarded as detached portions of the cœlom. The excretory organs of Nemertines lie between the vascular trunks and the exterior, and show no such relations with the cœlom.

Among the Annelids, on the other hand, the Chætopods possess an excretory system, which seems to present several features of strong resemblance with the nephridia of Vertebrates, and it remains to be considered whether an actual homology can be postulated in this case.

The points of similarity may for the present purpose be classed under seven heads. (1.) The Vertebrate and the Chætopod excretory systems agree in the fact that both primitively serve, at least in part, to convey to the exterior such fluids as accumulate in the cœlom, this cavity being used as a capacious excretory reservoir. (2.) In both groups certain portions of the epithelial lining of the cœlom become differentiated into specialized excretory glands. In Vertebrates the only structures of this character are the glomi and glomeruli; but in Annelids there is evidence that considerable areas of the peritoneum may become modified in this way. It was suggested by Claparède ('69, p. 615), that the chloragogen cells secrete certain elements from the blood and transfer them to the perivisceral fluid. This view of the function of the chloragogen cells has

been confirmed by a large number of observers,¹ and it has been further shown that individual cells, having become charged with excreted concretions, loosen from the layer to which they belong, and float freely in the coelom, whence they are discharged through the nephridia. The chloragogen layer covering the blood-vessels appears moreover from its anatomical relations to be a portion of the visceral mesoderm, and it has been shown to arise ontogenetically from that layer (Roule, '89, pp. 201, 252, 290). The chloragogen cells are frequently distributed upon special vascular processes, thus forming distinct glandular organs. Similar in function is probably the glandular envelope of the ventral vessel in *Polyopphthalamus* (E. Meyer, '82, p. 816), and cases may be found among *Polychaetes* in which definite peritoneal glands are present (Grobbe, '88, pp. 255 *et seq.*, Eisig, '87, pp. 227, 245, 681). I should not wish to assert a strict homology between the glomus and the masses of chloragogen cells; yet it seems to me likely that the latter represent an early differentiation of the splanchnic mesoderm of which we have more specialized developments both in *Annelids* and in *Vertebrates*.² (3.) The efferent conduits take their origin from the coelom by means of a series of ciliated funnel-shaped openings, the nephrostomes. (4.) The nephrostomes lead into transverse convoluted canals, along the course of which a large part of the excretion takes place. (5.) The nephridial tubes arise from the parietal peritoneum. (6.) They are typically strictly metameric, one pair of tubules being developed in each metamere. The deviation from this typical metamerism to which I have already referred in the case of *Vertebrates* is paralleled by similar conditions in *Capitella* (Eisig, '87, p. 594). (7.) The development of the *Chaetopod* nephridia resembles in general that shown by those of *Vertebrates*. Both the pronephric and mesonephric tubules arise as a series of metameric outgrowths from the somatic mesoderm. In *Polychaetes* this is evidently the mode of origin of the nephridia. In *Oligochaetes* the development is more doubtful, but the method of origin described by Bergh ('90) is in essence the same as that known in *Polychaetes*, and the mode of development maintained by Wilson ('87, pp. 185, 186, and '89, pp. 419 *et seq.*) may be interpreted so as not to be in fundamental opposition with such a method.

There is one feature in regard to which the nephridia of most *Anne-*

¹ E. g. Timm ('83, pp. 122, 123); Kükenthal ('85, p. 336); Meyer ('87, p. 648).

² A further analogy is possibly to be found in the fact that in *Amphibia* certain cells early loosen from the wall of glomus and fall into the coelom, leaving intervals between the remaining cells of the epithelium.

lids and those of Vertebrates differ conspicuously, viz. in the mode in which the nephridia terminate. It is a familiar fact, that in Annelids each nephridium opens separately on the surface of the body; while in Vertebrates the nephrostomal tubules all connect with a pair of longitudinal ducts opening into the cloaca. In commenting upon this feature of difference, it is important to note in the first place that the contrast is not of universal application. It has been recently shown by E. Meyer ('87, pp. 618-625) and Cunningham ('87^a, 87^b, pp. 248-253) that in *Lanice conchilega*, a terebelloid Annelid, certain nephridia open into a longitudinal trunk, and only secondarily communicate with the exterior. On the other hand, it is probable that *Amphioxus* possesses nephridia which open to the exterior (atria cavity) without the intervention of a longitudinal duct. If such differences can occur among the members of either group, it seems to me that it would be unjust to deny the homology of the other portions of the system in consequence of the fact that Vertebrates *in general* possess a longitudinal duct, while Annelids *in general* do not. It appears to me, moreover, that the condition of the nephridia in *Lanice conchilega* and the ontogeny of Vertebrates both serve to indicate the manner in which the duct may have secondarily arisen. In *Lanice conchilega* there is no doubt that the nephridial duct is a secondary growth, and it is highly probable that the channel is formed by outgrowths extending from each of the nephridial tubes backward and communicating with the next following nephridium. Two groups of nephridia can be distinguished in *Lanice conchilega*. The more anterior of these consists of a short longitudinal duct which bears three nephrostomal tubules, and terminates at its posterior end by a single pore. In the posterior set, the longitudinal duct is merely a canal which connects the several nephridia, while these continue to retain their external orifices. I have already pointed out that the ontogeny of Vertebrates presents a similar process in the development of the longitudinal canal of the pronephros, and have shown that such changes may likewise have taken place in the region of the mesonephros. I by no means wish to imply by this comparison a belief that the ordinary mode of development in Vertebrates is to be directly derived from that presented by *Lanice conchilega*, nor to assume a close genetic relation between Vertebrates and genera presenting this condition. I merely wished to emphasize the fact, that in *Lanice conchilega* we have an instance of a species which, primitively possessing discrete nephridia, such as may have been present in the ancestor of Vertebrates, has acquired a longitudinal excretory canal by a process of transforma-

tion which resembles that by which Vertebrates acquire in their ontogeny the segmental duct.

From the facts thus far brought forward, I conclude, (1) that the group of animals which presents nephridia most closely resembling those of Vertebrates is unquestionably that of the Chaetopod Annelids; and (2) that the Vertebrate excretory system can be readily derived from that of Annelids by a series of steps which are in accord with the evidence afforded by the ontogeny of Vertebrates.

In conclusion, I shall briefly allude to the opinions of previous writers respecting the origin of the Vertebrate excretory organs. These opinions fall, in the first place, into two classes, according to one of which the excretory system is derived from Invertebrate ancestors; according to the other, it has arisen wholly within the Vertebrate phylum.

The most recent exponent of the latter view is van Wijhe ('89, pp. 506 *et seq.*). The arguments offered by this author in support of his position are in part dependent upon his denial of the serial homology of pronephros and mesonephros. Van Wijhe also employs two arguments which are independent of his position in regard to this point: (1) nephridia are absent in Amphioxus, and therefore the common ancestral form cannot have possessed them; (2) the renal organs do not appear until after the so-called "Acrania stage," and therefore could not have appeared phylogenetically until this stage had been passed. Granting both premises, it seems to me that neither conclusion follows. With reference to the absence of kidneys in Amphioxus, the possibility—or should I not say probability?—of extensive degenerative modification is entirely neglected. Moreover, it has now been rendered probable that true nephridia do exist in Amphioxus, an observation which removes at a blow the whole basis of the argument. I do not believe many embryologists would unite with van Wijhe in holding that characters which appear simultaneously in the ontogeny of a form must necessarily have arisen contemporaneously in its ontogeny. For my part, I am unable to diagnose with accuracy the "Acrania stage" of Amphibia; but Ostroumoff ('88, pp. 77, 78) appears to have had the necessary insight, and denies emphatically that the pronephros in the case of Reptiles arises after the "Acrania stage."

Turning now to the hypotheses that have been advanced involving the derivation of the Vertebrate excretory system from Invertebrate ancestors, Haeckel ('74, p. 37), Gegenbaur ('78, p. 628), and Fürbringer ('78^a, pp. 95 *et seq.*) endeavored to show that the Vertebrate nephridia were derived from those of Plathelminthes. Semper and Balfour, on the

other hand, claimed that the transverse tubules of the mesonephros are homologous with the segmental tubes of Annelids, an opinion which is shared by Beard, Haddon, Kollmann ('82^a) and others.

Rückert ('88, p. 262) has recently denied this homology, and asserted that the Annelidan nephridia are represented in the pronephros alone, since the latter is the only portion of the system of metameric tubes which comes in contact with the ectoderm. With Rückert, I would admit that the evidences of an Annelidan origin of the excretory system are to be sought mainly in the pronephros; but, in view of the intimate relations which exist between pronephros and mesonephros, it seems to me more probable that both have had the same phylogenetic origin. Ostroumoff ('88, pp. 80 *et seq.*) also has asserted that the development of the pronephros indicated that this system had been inherited from Annelidan ancestors.

On the other hand, a number of authors maintain that, although the mesonephros may be derived from the segmental organs of Annelids, the pronephric system is represented by other Invertebrate nephridia. Thus, Semper ('76, pp. 387, 388) suggests the possibility that the duct (pronephric system so far as present in Selachians¹) may represent the unsegmented excretory tubules developed in the larva of Nephelis, or may even represent an inheritance from the Plathelminthan water-vascular system; and Balfour ('81, p. 607) was led to accept this view.

In addition to his hypothesis of an origin of the excretory system of Vertebrates from that of Plathelminthes, Fürbringer suggests the possibility that the entire system may have arisen from Gephyreans, in which unsegmented and segmented excretory organs are stated to coexist. This view was adopted by Kollmann ('82^a), and is offered as a suggestion in Wiedersheim's ('86, p. 731) text-book.

None of the views which claim a *double* origin for the excretory system seem to me to be tenable, in consequence of the fact that the pronephros, an undoubtedly segmented element, develops in *strict continuity* with the duct, the so-called unsegmented element. The view according to which the excretory system is derived from that of Gephyreans, moreover, is liable to special criticism. This claim that Gephyreans present an excretory system of a double nature, segmented and unsegmented, doubtless refers to the coexistence of two or three pairs of nephridia, together with the so-called Analschläuche of Echiurids. I am unable to see the slightest evidence that the Vertebrate excretory system is made

¹ By this suggestion, Semper allows some justification to W. Müller's conception of a pronephros, although he had earlier contested it.

up of such remote parts; and the hypothesis must fall to the ground as soon as it is proved that the Analschläuche are in reality only modified nephridia opening into a proctodæum. In addition to the anatomical evidence favoring such an interpretation, Hatschek ('80, pp. 60-62) has recently advanced strong evidence from an embryological standpoint for believing that they are nephridia which primitively opened directly to the exterior.

Among the attempts to find a homologue of the segmental duct already present in the Worms may be mentioned the longitudinal canal described by Hatschek ('78, p. 117 *seq.*) in the larva of *Polygordius*. Were Hatschek's account accurate, it would doubtless warrant great changes in our conceptions of the interrelationships of the Vernian nephridia, and of the origin of the Vertebrate kidneys; but his statements have not been confirmed by any subsequent observations, though several investigators have concerned themselves with this interesting form (Fraipont, '87, p. 83; Eisig, '87, p. 662; E. Meyer, '87, p. 594; Bergh, '85, p. 27, footnote).

The remaining views in respect to the nature of the duct agree in regarding it as a secondary growth.¹ Beard ('87, p. 651) and Haddon ('87) accept the ectodermal origin of the segmental duct, and endeavor to give it phylogenetic significance by assuming that the ontogenetic connection of the duct with the ectoderm indicates that it was represented in the phylogeny at first by a groove into which the nephridia opened, and that this groove gradually became closed and was cut off as a tube extending from the most anterior tubule backwards to the cloaca. Rückert ('88) and van Wijhe ('89, pp. 507, 508), on the other hand, assert a gradual backward growth of a primitively anterior opening.

None of these views are compatible with the fact that in many Vertebrates the duct is demonstrably of mesodermal origin. They also seem to me to give insufficient significance to the mode in which the pronephric diverticula unite to form a longitudinal canal; and the first two authors neglect it wholly.

Finally, the abandoned view of Balfour ('76, pp. 25, 26), according to which the duct arises by the fusion of the distal ends of the several nephridia, has been revived by Eisig ('87, p. 649), and was accepted by Rückert ('88, p. 264) for the pronephric portion of the duct. This view seems to me much the most probable, and I am inclined to accept it.

¹ Here belongs also the view of Boveri ('90), which has already been discussed (page 265).

In the foregoing discussion, I have endeavored to show that there is considerable evidence in favor of the view that the excretory system of Vertebrates has developed from a system of metameric nephridia, such as are present in Annelids. None of the evidence seems to me, however, final. The excretory systems of the two groups are very similar, but we have no means of limiting definitely the part that has been played by physiologically similar needs in moulding the structure of the organs. Nor am I committed to the theory of the Annelidan extraction of Vertebrates. I fully realize that such a theory can only be established by investigations which shall include in their scope the entire organization of the two groups. So far as this larger theory has been dealt with in this discussion, it has been with the view of contributing such evidence as the excretory system offers, and I have naturally left untouched the mass of evidence which proceeds from other organs. To this in addition we must appeal for the justification of the broader theory.

CAMBRIDGE, April 25, 1891.

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